

Unravelling the Importance of Benthic Mineralisation and Nutrient Cycling in Macquarie Harbour, Tasmania

By

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Declaration

Statement of originality

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Abstract

Sediment biogeochemistry was studied in Macquarie Harbour, which is located on the west coast of Tasmania, Australia. Rates of respiration and fluxes of nutrients, which are either released or taken up during mineralisation of organic matter, were investigated at a series of harbour wide sites and at sites under and adjacent to fish farms. Environmental characteristics of the sediments and overlying water column were also measured to examine their role in determining sediment biogeochemistry of the harbour. The purpose of this study was to understand benthic mineralisation and nutrient cycling, particularly nitrogen cycling, in Macquarie Harbour and the environmental controls of these processes. This study also assessed the influence of organic loading from fish farming activities on sediment biogeochemistry.

Sediments cores were collected from nine sites across the harbour in November 2012; these sites were located well outside the influence of benthic enrichment from fish farms. Sediments were also collected from nine other sites to examine the influence of organic enrichment from fish farming; six sites were located directly under the outer edge of fish cages and three sites 50 m from the edge of the cages. To investigate the influence of farm management practices, the cages, 50 m from cage and a subset of the control sites were sampled again in January, May and September 2013.

The harbour wide study showed that the sediments in Macquarie Harbour contained mainly terrestrial sourced organic matter based on its depleted carbon signatures. Characterisation of the organic matter also showed gradients commonly observed in estuaries where the isotopic signatures of carbon and nitrogen increased and the percentage of total organic carbon and total nitrogen contents generally decreased from the upper towards the lower estuary.

Oxygen consumption and dissolved inorganic carbon (DIC) production rates suggest that aerobic respiration were the dominant respiration pathway except at a few sites where anaerobic respiration were prevalent. The uptake of nitrate from the water column for denitrification at the majority of sites suggest that uncoupled nitrification-denitrification was an important anaerobic pathway in the harbour. The release of ammonium at most of the sites further suggest that nitrification in the sediments were limited, most likely due to low oxygen penetration of sediments. Multiple regression of individual fluxes shows that oxygen was a clear controlling factor for benthic mineralisation and nutrient cycling in Macquarie Harbour.

The influence of fish farming on the benthic processes was clearly evident. Oxygen consumption was six times higher at cage sites compared to the control sites. While oxygen consumptions at 50 m from the cage sites were often elevated compared to the control sites, the rates were not significantly higher. The high DIC/O₂ consumption ratio, especially at the cage sites, indicates that the main degradation pathway was through anaerobic respiration. The presence of a white filamentous mat on the sediments taken from the fish cages observed during the experiment highlight the likely role of anaerobic sulfate reduction.

Ammonium fluxes from sediments into the water column were also significantly higher at the fish cages and conversely, nitrate uptake from the water column were higher at cage sites. This is consistent with a reduction in sediment nitrification due to increased bacterial respiration and low oxygen concentrations and a greater dependence on nitrate from the water column for denitrification (i.e. uncoupled nitrification-denitrification). There was also evidence that some of the nitrate is reduced to ammonium via dissimilatory nitrate reduction to ammonium (DNRA). The release of phosphate from the sediments occurred at the cage and 50 m sites, in contrast to phosphate uptake at control sites. Despite the observed higher ammonium release from farm enriched sediments, there has been no

significant change in bottom water ammonium concentrations during the period of significant industry expansion based on data from the monthly water quality monitoring program. This suggests that more broadly across the harbour, nitrification in the water column and sediment denitrification may be buffering the system against the increased loads. This remains to be tested.

Based on the flux rates, there was no clear indication that there was any temporal changes in Macquarie Harbour. This may reflect relatively stable bottom water conditions throughout the year more broadly in the harbour. Sediment conditions showed improvement at some of the cage sites during fallowing. However, the sediment response to fallowing at some areas was not as expected. In one of the leases, benthic flux rates did not show signs of recovery during fallowing (i.e. lower benthic respiration rates, lower nutrient flux rates), and conversely, during stocking the flux rates declined instead. This suggest that other factors are likely to be responsible for organic loading and mineralisation of organic matter.

This study is the first assessment of benthic nutrient cycling in Macquarie Harbour sediments. Importantly it describes sediment function more broadly in the harbour and in response to organic enrichment due to fish farming. The more recalcitrant organic matter brought in by the rivers has kept the background oxygen consumption and nutrient levels low. Despite low oxygen consumption, bottom water oxygen concentrations are naturally low due to the long residence times and reduced mixing with surface waters. Sediment nitrification appears to be reduced as a result, with sediment denitrification relying on nitrate sourced from the water column. Lastly, considering that oxygen concentration is an important driver of sediment function in Macquarie Harbour, the recently observed decline in bottom water dissolved oxygen concentration and its implication for nutrient cycling warrants attention.

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Chapter 1: Introduction

General Introduction

Estuaries are important transition zone connecting land, freshwater and marine environment. Not only are estuaries high in productivity and biotic diversity, these systems also provide critical habitat for commercially important fishes and shellfish (Bianchi, 2006). The estuarine and coastal environment supports many human uses (Kennish, 1994) for instance as a place for recreational activities, and infrastructure such as ports for shipping and transportation and fisheries. Growing human populations have also increased urbanisation along the coastal waters, escalating the extent of disturbances in the coastal environments (Small & Nicholls, 2003). Some of these human activities, for instance industries, agriculture and municipal sewage have caused anthropogenic problems such as nutrient enrichment, declines in water quality and impacts on the diversity of marine life (Howarth et al., 2000; Howarth et al., 2011; Kennish, 1994). The ecological processes and functions of the receiving environment provide important ecological services which includes organic matter decomposition, nutrient cycling, primary and secondary production and pollution transport and transformation (Levin et al., 2001). However, continuous inputs from anthropogenic sources to the estuarine environment could potentially change the ecological processes which are important for the recycling of nutrients, oxygen regulation in water and sediments and early diagenetic processes (Middelburg et al., 1993).

Benthic estuarine environments are sites where processes such as mineralisation of organic matter takes place. The source of organic matter are primarily plant materials and animal faeces (Bianchi, 2006). Mineralisation of organic matter is an important process for the recycling of nutrients which are continuously used for primary production. With anthropogenic activities supplying additional nutrients, increased rates of primary production may result with a greater potential for algae blooms, and thus eutrophication (Cloern, 2001; Paerl et al., 2006). The subsequent increase in the supply of organic matter

will increase microbial respiration rates and this can lead to hypoxic and anoxic condition (Cloern, 2001; Middelburg & Levin, 2009; Rabalais et al., 2002).

Deposition of organic matter onto the estuary floor undergoes mineralisation either through aerobic or anaerobic respiration (Jørgensen, 1983; Kristensen, 2000). Aerobic respiration is usually dominant in the oxic zone of the sediment where oxygen is the preferred terminal electron acceptor (Bianchi, 2006). This process mineralises organic matter to H_2O , CO_2 and inorganic nutrients. As the oxic zone typically only extends to a few mm in coastal sediments, mineralisation of organic matter that is buried deeper in the anoxic layer of the sediment is carried out through anaerobic pathways. Anaerobic mineralisation occurs in a sequence of terminal electron receptors by different types of bacteria. The order starts from nitrate (NO_3^-), manganese oxide (MnO_2), iron oxide ($\text{Fe}(\text{OH})_3$), sulfate (SO_4^{2-}) and CO_2 (Figure 1) (Bianchi, 2006; Canfield, 1993). An increase in the deposition of organic matter will first lead to an increase in aerobic respiration and the rapid consumption of oxygen. When the supply of oxygen becomes limiting, anaerobic respiration becomes dominant in highly enriched sediments.

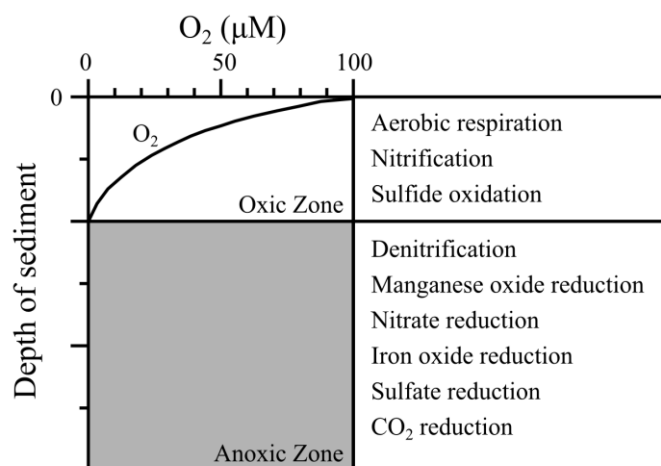


Figure 1 The theoretical vertical distribution of diagenetic processes in marine sediment. The depth scale is an approximate (modified from Kristensen, 2000).

During sediment mineralisation, the release of nutrients is imminent. One of the nutrients that is of particular interest in the estuarine and coastal environments is nitrogen as it is one of the nutrients that are limiting to primary productivity (Howarth & Marino, 2006). Ammonium (NH_4^+) is released via aerobic respiration through the process of ammonification. The ammonium produced may have several fates which include release to the overlying water, adsorption on to sediments, assimilation by plants or bacteria, or transformation to nitrogen gas (N_2) via the process of ammonium oxidation (otherwise known as Anammox) or oxidation to nitrite (NO_2^-) then nitrate (NO_3^-) via the process of nitrification (Blackburn & Henriksen, 1983; Cornwell et al., 1999; Joye & Anderson, 2008). Together with ammonium, nitrite and nitrate can also be released into the overlying water and these bioavailable forms of nitrogen therefore add to the broader supply of nutrients available for primary production. However, the nitrate and nitrate formed via nitrification may also be transformed via the process of denitrification which permanently removes nitrogen from the environment as nitrogen gas. Denitrification is mediated by anaerobic bacteria that use NO_3^- as a terminal electron receptor (Cornwell et al., 1999). Denitrifying bacteria may use NO_3^- obtained either from nitrification in the sediment or from the water column. When the microbial process involves the former, the process is referred to as coupled nitrification-denitrification whereas the use of nitrate from the water column is referred to as uncoupled denitrification (Herbert, 1999).

Nitrogen may also be retained in the form of NH_4^+ produced via the process of dissimilatory nitrate reduction to ammonium (DNRA). This process occurs in the presence of high sulfide concentrations in the sediment (An & Gardner, 2002; Gardner et al., 2006). Both processes, denitrification and DNRA, are mediated by bacteria that couple the oxidation of organic carbon or reduced iron and sulfide to the reduction of NO_3^- to NH_4^+ or N_2 (Hulth et al., 2005) in the anoxic zone and also compete for NO_3^- as an electron acceptor.

The broader understanding of benthic nutrient cycling has improved through experiments and conceptual models, however knowledge at a local scale is still required to predict and manage any disturbances, such as changes in organic enrichment, to the environment.

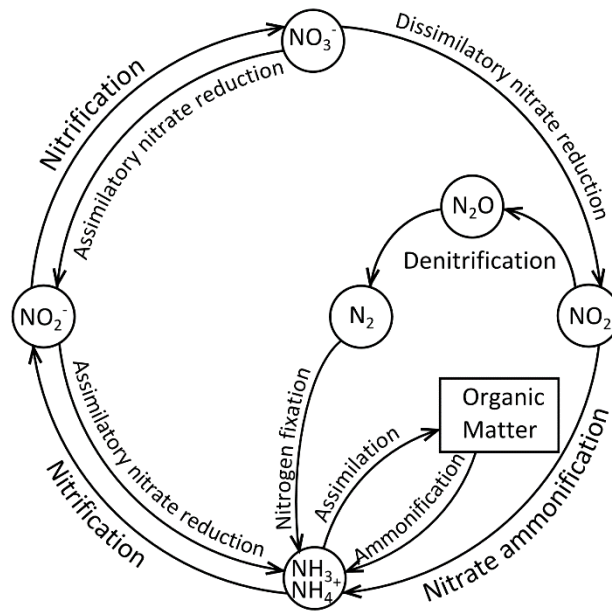


Figure 2 The chemical species and major processes in the biogeochemical cycling of N (taken from Herbert (1999) without modification).

The rates of benthic respiration and nutrient fluxes can be influenced by several factors. This includes organic loading (Caffrey et al., 1993; Christensen et al., 2000; Holmer & Kristensen, 1992), presence of macrofauna (Glud et al., 2003; Heilskov & Holmer, 2001; Kristensen, 1988, 2000) and oxygen concentration (Glud, 2008; Middelburg & Levin, 2009). As discussed, an increase in organic matter supply to sediments increases respiration rates and nutrient regeneration. However, the depth of the water column also needs to be considered given that mineralisation of organic matter also occurs in the water column before it reaches the sediment (Boynton & Kemp, 2008).

Oxygen concentration can also influence benthic respiration and nutrient flux rates. Processes such as aerobic respiration, nitrification and denitrification depends on the availability of oxygen in the overlying water column and sediment. Oxygen concentration is

known to regulate denitrification rate as oxidation of ammonium either in the overlying water column or sediment is needed to start the process of denitrification (Rysgaard et al., 1994; Voss et al., 2011). A reduction in oxygen concentration could also cause a shift from aerobic respiration towards anaerobic respiration (Middelburg & Levin, 2009). The presence of macrofauna can also significantly affect the processes in sediments. Bioirrigating macrofauna can stimulate mineralisation of organic matter and nutrient flux rates by increasing the oxygen flow into the sediments (Kristensen, 2000). Organic matter can also be transported between the aerobic and anaerobic zones within the sediments which leads to greater exposure of organic matter for mineralisation (Aller, 1994).

The enhanced input of organic matter is not limited to the supply from primary production. Finfish aquaculture is known to enrich benthic sediments in coastal and estuarine environments through the production of organic waste in the form of uneaten feed and fish faeces. The future demand for aquatic resources are only increasing with growing human populations (Bianchi, 2006) and aquaculture has become one of the world's major food production industries (FAO, 2014). According to the Stephan and Hobsbawn (2014), salmonids (salmon and trout), which are mostly farmed in Tasmania, have been Australia's most valuable seafood product since 2004-05. The increased use of coastal areas for farming and intensified farming production in Tasmania has given rise to awareness of potential environmental problems associated with fish farming. Therefore, increased effort is put into planning and regulation to ensure that fish farming is environmentally sustainable.

Atlantic salmon take approximately 2 years to grow to harvest size in culture based on a diet of nutrient rich feed that generally contains fishmeal, plant protein product, animal by-product meal, crustacean meal or other food additives for growth and aesthetic value. Over the last 30 years, feeding practices have improved significantly with the improvements of feed conversion ratio and the use of automatic mechanical feeders (Belle & Nash, 2008).

However, the loss of unconsumed feed is inevitable and combined with fish faeces, can form a significant source of organic enrichment on the seafloor beneath pens. The distribution of waste material is normally concentrated beneath the pen (Valdemarsen et al., 2009) nevertheless it also depends on the depth and current speed of the farming area (Black et al., 2008). Greater depth and stronger current result in a larger depositional footprint but lower degree of enrichment whereas weaker currents cause a greater accumulation of deposits closer to the pen (Black et al., 2008). Nutrients from fish excretion (ammonium, urea, etc.) are generally dispersed and diluted by surface currents. However, together with mineralisation and the release of nutrient from locally deposited organic enrichment, it contributes to the broader source of nutrients released from salmon farming that is available for primary production. To ensure sustainable management of fish farms and ecosystem protection from nutrient enrichment, activities such as fallowing of sediments is important. Fallowing is a process of leaving areas for seabed free from farming, allowing the seabed to eventually recover. The fallowing period may vary at different farm sites due to conditions of the system such as residence time, rates of benthic processes, magnitude of impact etc., to effectively reduce the probability for eutrophication to occur.

Growing global and domestic demands for salmon inevitably triggers the need for expansion, and thus the search for new coastal areas suitable for farming. In Tasmania, Macquarie Harbour offers a vast area suitable for salmon aquaculture. However, this estuarine system is unique. The presence of a sill at the mouth of the estuary causes long residence times and the large catchment inputs of freshwater result in a highly stratified water column year round. This means that bottom water oxygen concentrations are naturally low due to limited input of oxygenated oceanic waters. Similarly, vertical mixing is limited due large freshwater inputs and the highly stratified water column. Thus it is important that

farm inputs of organic matter are managed such that the biological oxygen demand of farm wastes doesn't exceed the rate of oxygen renewal.

Christensen et al. (2000) studied the effect of fish farming in an estuarine fjord on nitrogen processes. The study found that DNRA was high directly below fish cages compared to denitrification while DNRA was negligible at sites unaffected by fish farming. They estimated that only 0.1% of the nitrogen input from fish farming was removed by denitrification and with the majority of nitrogen released to the water column as ammonium. In another study, Nguyen et al. (2012) discovered that fish farming activities in Nha Phu Estuary, Vietnam led higher respiration rates and the elevated release of ammonium. Sediment nitrification was also inhibited and denitrification rates were negligible which means that the estuary retained nitrogen that could be a source for pelagic primary production. These studies demonstrate that important nitrogen transformation processes, such as nitrification, DNRA and denitrification, are impacted in enriched sediments below cage aquaculture, and as a result, the amount of bioavailable nitrogen that ultimately enters the system. The depletion of oxygen is also known to intensify processes such as sulfate reduction of organic material. Although the effects of benthic enrichment from cage aquaculture have been well documented in these study areas, interpretation and application of these predictions to other farming locations is tenuous. The physical, biological and chemical characteristic of each system are unique, and therefore, local measurements and data are imperative to ensure a robust understanding of benthic responses. Given the unique conditions of Macquarie Harbour, understanding the interaction of benthic processes is crucial.

This study was driven by the lack of understanding of benthic processes in Macquarie harbour in the face of the proposed expansion of fish farming. The aims of this study were to 1) quantify benthic respiration and nutrient flux rates at system and farm

scales, 2) identify the relationship between benthic processes and environmental drivers, 3) obtain preliminary information on benthic-pelagic interactions in Macquarie Harbour, and 4) investigate the effects of fish farming on benthic respiration and nutrient processes.

The three chapters are presented with the following objectives, with Chapters 2 and 3 written in a style suitable for publication:

- 1) Chapter 2: Quantifying the benthic processes and identifying potential environmental drivers at a system scale. Comparison to previously studied estuaries.
- 2) Chapter 3: Quantifying and understanding the benthic response due to organic enrichment from fish farming. Preliminary assessment of benthic processes in response to farm management.
- 3) Chapter 4: Synthesis of results from both chapters and discussion of the major findings, particularly in relation to nitrogen cycling and availability and the influence of oxygen on Macquarie Harbour's benthic processes.

Chapter 2: Spatial variation in benthic fluxes of respiration and nutrients in Macquarie Harbour

Abstract

Benthic studies were conducted using sediments collected from various sites around Macquarie Harbour, Tasmania, Australia. Fluxes of dissolved oxygen, ammonium, nitrate, nitrite, phosphate, dissolved inorganic carbon (DIC), denitrification and dissimilatory nitrate reduction to ammonium (DNRA) were measured for nine sites with depths ranging from 7 to 40 m. The isotopic signatures of the sediment showed a gradient with increasing carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) signatures from the upper to the lower reaches of the harbour. Respiration rates were lower around the central harbour which could be attributed to several factors including low dissolved oxygen in the overlying water, recalcitrant organic matter and depth. The DIC: oxygen ratios were >1 at four sites indicating anaerobic respiration at those sites. The sediments in the harbour were generally a source of ammonium but a sink for nitrate. Nitrate uptake occurred at most sites. This nitrate might be used for either uncoupled denitrification or DNRA, consequently, uncoupled denitrification could play an important role in controlling nitrogen in the water column in Macquarie Harbour. Bottom water oxygen concentration was the most significant driver of the observed benthic fluxes, suggesting that changes in bottom water oxygen concentration could affect the sediment biogeochemistry in the harbour.

Introduction

Important ecological processes such as decomposition, nutrient cycling, primary and secondary production occur in estuaries (Alongi, 1998; Levin et al., 2001). Interaction between these processes can generate high productivity, making estuaries both ecologically and economically important (Barbier et al., 2011). Nutrient regeneration in sediments plays a key role in estuarine productivity where pelagic and benthic processes are strongly coupled

through the fluxes of nutrients across the sediment-water interface (Cowan et al., 1996; Herbert, 1999; Kemp & Boynton, 1984).

One of the most important processes in coastal nutrient processing is the nitrogen (N) cycle; understanding the transformation and fate of N is important because it is a limiting factor for primary production (Howarth et al., 1988; Ryther & Dunstan, 1971). In sediments, mineralisation of organic matter produces ammonium (NH_4^+) (Bianchi, 2006) which can then be released from the sediment into the overlying water column, adsorbed onto sediments, assimilated by plants or bacteria, oxidised to nitrite (NO_2^-) then nitrate (NO_3^-) (nitrification) or transformed to N_2 via the process of ammonium oxidation (Cornwell et al., 1999; Joye & Anderson, 2008). Denitrification takes place in deeper anoxic sediment layers and converts NO_3^- , sourced either from nitrification or from the overlying water column, to gaseous nitrogen (N_2), effectively removing the N from the system (Cornwell et al., 1999; Rysgaard et al., 1994). However, dissimilatory nitrate reduction to ammonium (DNRA) can also occur in the anoxic sediment layers, in this case N is retained in the system when NO_3^- is reduced to NH_4^+ (An & Gardner, 2002).

The cycling of phosphorus (P) between the sediment and water column is also important in coastal water bodies, and can in some systems limit primary productivity. The fate of phosphate (PO_4^{3-}) in the sediments is largely controlled by the presence of iron hydroxide which has the capacity to adsorb PO_4^{3-} , releasing it again when iron (III) is reduced to iron (II) in anaerobic conditions (Kraal et al., 2015; Roden & Edmonds, 1997). The release or retention of nutrients, such as PO_4^{3-} and NH_4^+ , produced from mineralisation of organic matter in the sediments, can influence both primary productivity and re-supply of organic matter.

In coastal areas, increased nutrient loading from anthropogenic activities has caused an increase in eutrophication (Howarth et al., 2000). Eutrophication, which is defined as an increase in the rate of organic matter supply in an ecosystem (Nixon, 1995), stimulates benthic respiration which rapidly consumes oxygen (Howarth et al., 2000; Jensen et al., 1990; Trimmer et al., 2000). Prolonged rapid oxygen consumption leads to hypoxia (< 2.0 mg/L), a common side-effect of eutrophication (Diaz & Rosenberg, 2008). The increase rate of respiration due to an increase in the supply of organic matter not only decreases oxygen concentration, it also supplies more nutrients to the water column which can further fuel primary production.

Denitrification is an important mechanism for the removal of N (Seitzinger, 1990). However, the importance of denitrification differs among systems in response to changes in the concentration of water column NO_3^- , rates of carbon mineralisation, oxygen concentration of the overlying water, oxygen penetration depth and the presence or absence of vegetation and macrofauna (Cornwell et al., 1999; Nixon et al., 1996). At the same time, some of these controlling factors are not just limited to regulating denitrification. Oxygen concentration in the sediment and overlying water also has the potential to change other biogeochemical processes given that oxygen has a significant influence on the various processes that underpin both the N and P cycles (Joye & Anderson, 2008; Rysgaard et al., 1994; Sundby et al., 1992). In the case of denitrification, oxygen levels regulate the rate of denitrification, influencing the oxidation of NH_4^+ (Rysgaard et al., 1994; Voss et al., 2011). A reduction in oxygen concentration could also cause a change in microbial communities that use alternative electron acceptors such as NO_3^- , manganese (IV), iron (III) and sulfate (Middelburg & Levin, 2009). Hypoxia is not limited to anthropogenic-induced eutrophication, it can also be the result of natural or a combination of natural and anthropogenic-induced processes (Middelburg & Levin, 2009). Changes in hydrodynamics

such as water column stratification, restriction in water exchange and high residence times can also decrease oxygen levels and lead to hypoxia or anoxia (Diaz & Rosenberg, 2008; Eyre, 1998; Rabalais et al.).

There are a range of other factors that could influence biogeochemical processes, for example the burrowing activities of macrofauna can influence mineralisation and sediment nutrient fluxes by introducing newer organic matter deeper into the sediment and providing a new interface for microbial colonisation (Kristensen, 1988). Nizzoli et al. (2007) found that *Nereis* bioturbation stimulated oxygen and NH_4^+ fluxes between sediment and water column, and uncoupled denitrification. Physical properties such as sediment grain size can influence solute transport between sediment and the water column. For example, the rate of oxygen and nutrient exchange between the sediment and overlying water column will be influenced by the type of transport, either through diffusive transport common in fine sediment or through advective pore-water transport which is common in larger sediment grain size (Cook et al., 2007; Huettel et al., 2014). The combination of factors that are likely to be important in influencing biogeochemical processes in the sediments vary from system to system, highlighting the importance of local understanding of biogeochemical processes.

Macquarie harbour is a unique estuary with fjord-like hydrodynamic characteristics. The harbour has been studied comprehensively over the past four decades as a result of mining discharge and dam activities, which have caused changes to the harbour. Several studies have provided information on the hydrology, hydrodynamic, biological and sediment chemistry of the harbour as part of rehabilitation efforts and scientific research (see Carpenter et al.; Cresswell et al., 1989; Koehnken, 1996; O'Connor et al., 1996; Talman et al., 1996; Teasdale et al., 1996; Tong & Williamson, 1998). Since mining activities ceased in the 1990's, the key industries associated with Macquarie Harbour are tourism and aquaculture of salmon and trout. The salmonid industry is rapidly expanding in Tasmania,

with a particular focus on Macquarie Harbour, where the water quality conditions such as a thick freshwater layer, low rates of fouling and low seal activity are particularly suitable for growing salmon (DPIPWE et al., 2011). However, there is potential for negative effects associated with fish farming due to the inputs of dissolved nutrients to the water column and particulate loads to the benthos associated with faeces and uneaten feed (X. Wang et al., 2012). Given the unique characteristics of the harbour environment, such as naturally low bottom water dissolved oxygen conditions (Koehnken, 1996) and low light penetration in the water column (O'Connor et al., 1996), the system response may not be easily predicted from our understanding of conditions (Koehnken, 1996; O'Connor et al., 1996; X. Wang et al., 2012) in other systems. There is a lack of understanding of the benthic biogeochemical processes in Macquarie Harbour, addressing this knowledge gap is important particularly if we wish to understand the implications of increased benthic enrichment associated with further expansion. Therefore, the key aims of this study are to, for the first time, quantify the rates and processes of benthic mineralisation of organic matter in Macquarie Harbour and to determine the environmental drivers that could influence these processes. This information will provide critical background understanding to underpin the assessment of the impacts of farm based enrichment on benthic processes addressed in Chapter 3.

Materials and Methods

Study location

Located on the West Coast of Tasmania, Macquarie Harbour is an estuary with a deep central basin with depths of 30 to 55 m. The harbour receives water from three major sources: freshwater from the Gordon and King rivers and seawater from the open ocean (Koehnken, 1996). A sill at the inlet of the harbour restricts the exchange of water between the harbour and the open ocean, a characteristic commonly found in fjords. The restricted

water exchange has resulted in a stratified water column with high dissolved oxygen (DO), low salinity surface waters; low DO and higher salinity bottom waters caused by long residence time and an intermediate layer which is a mix of the bottom and surface layer (see Carpenter et al., 1991; Cresswell et al., 1989; Koehnken, 1996).

The harbour, along with other estuaries on the western and southern coast of Tasmania, are considered to have low productivity compared to other estuaries in Tasmania (Edgar et al., 1999). Edgar et al. (1999) attributed this to low inputs of dissolved nutrients and the dark tannin waters. For example, the abundance and diversity of macrofauna in Macquarie Harbour is considered low (Edgar et al., 1999; O'Connor et al., 1996). Other than small areas of reef found below 15 m depth in Macquarie Harbour, approximately 77% of the total substrate area is predominantly silt and 19% is sand, with the sandy areas generally located around the inlet at Hells Gate (Lucieer et al., 2009).

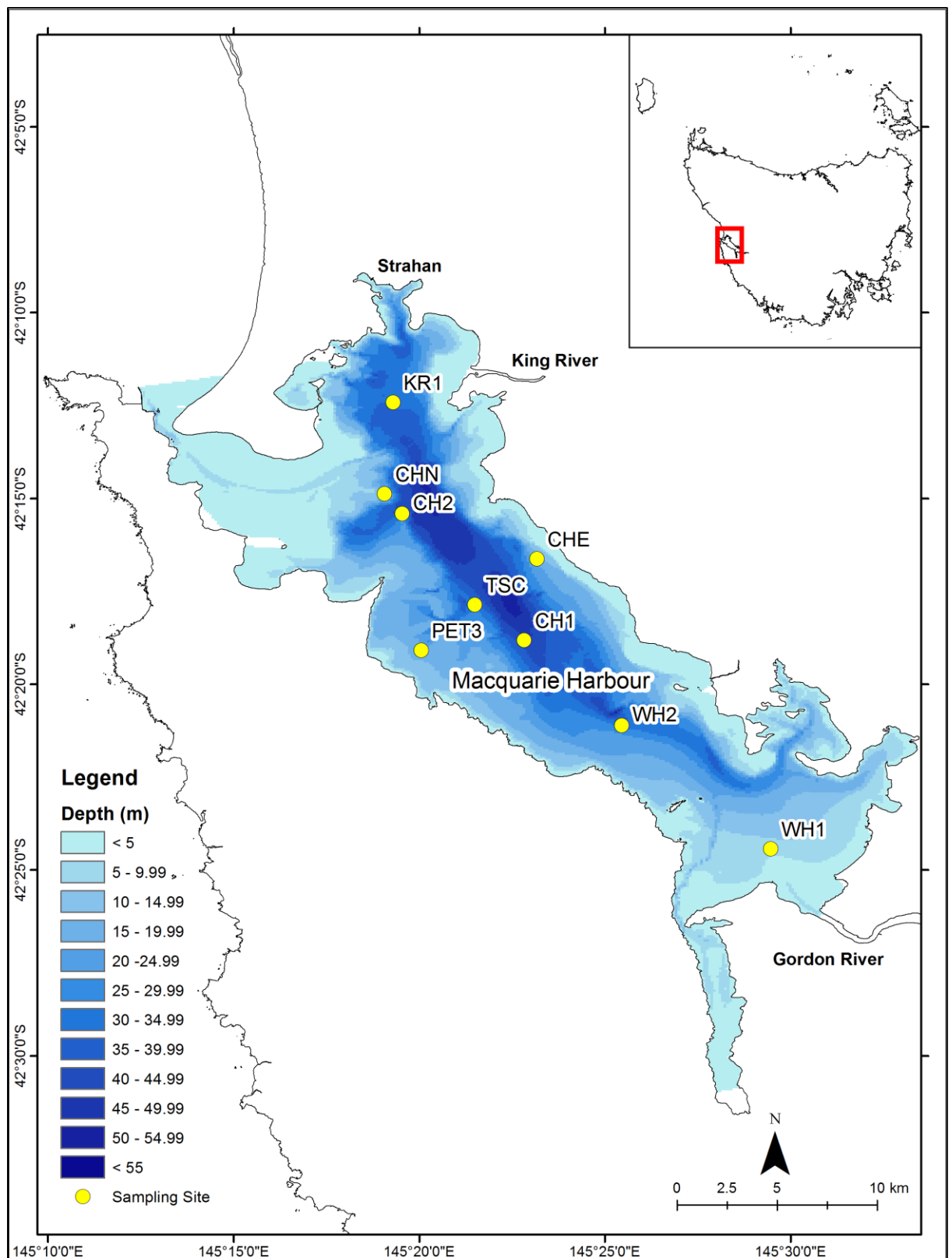


Figure 3 Map of study sites.

Sample collection

To examine benthic mineralisation at different locations in the harbour, nine sampling sites were selected as shown in Figure 3 with location details provided in Table 1. The sampling sites were chosen to include a broad coverage of the harbour and to overlap with water quality monitoring sites used as part of the Fish Farm Environmental Monitoring Program (FFEMP) (DPIPWE, 2012; DPIPWE et al., 2011). Using a box corer with a Perspex liner attached, four box cores containing undisturbed sediment were collected from each site in November 2012. Sediments from each box core were subsampled using two different sizes of polyethylene cylindrical cores for benthic flux analysis. For benthic respiration and nutrient flux (oxygen (O_2), dissolved inorganic carbon (DIC), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-})) measurements, a large core measuring 300 mm and 150 mm in diameter was used. A smaller core 300 mm and 44 mm in diameter were used for measuring denitrification and dissimilatory nitrate to ammonium (DNRA) flux rates. All the cores were filled with sediments to a depth of around 100 mm. Without disturbing the sediments inside, the cores were filled with bottom water taken from approximately 1 m above the sediment in the harbour and then stored in a tub filled with bottom water prior to transfer to the laboratory. Measurements of bottom water temperature, salinity and DO concentration were also taken using a YSI 6600 V2 Multi Parameter Water Quality Sonde with a YSI 650 MDS logger (YSI Incorporated, Ohio, USA).

Table 1 Name and code, with coordinates of each sampling site. To avoid confusion, the site names and codes used in this study are identical to the ones used in the FFEMP.

Site Name	Site Code	Coordinates (GDA 94)	
		X	Y
World Heritage Site 1	WH1	375844.8	5303913.08
World Heritage Site 2	WH2	370224	5309960.28
Central Harbour Site 1	CH1	366536.4	5314142
Central Harbour Petuna	PET3	362733.8	5313568.72
Central Harbour Tassal	TSC	364678	5315867
Central Harbour East	CHE	366930	5318194
Central Harbour Site 2	CH2	361896.3	5310351.61
Central Harbour North	CHN	361224	5321325
King River	KR1	361454.8	5325884.75

Sediment-water nutrient fluxes

The sediment cores of each sampling site were first immersed in a plexiglass tank filled with bottom water drawn from the sampling site. Site water was used to emulate local conditions of the sediment-water interface. The top of the cores were left open and stirred overnight in a temperature controlled water bath, which is used to maintain *in situ* water temperature, to equilibrate. Each core was stirred at a rate adequate to allow mixing of the water column inside the cores as well as to prevent resuspension of the sediments. All incubations were conducted in the dark, to reflect the dark tannin waters of the harbour. Once the cores were sealed to begin incubation, 130 ml of water samples were taken for dissolved nutrients (NH_4^+ , NO_2^- , NO_3^- and filterable reactive phosphorus (measured as PO_4^{3-})) and DIC analyses using plastic syringes. All samples were filtered through a 0.45 μm filter (Minisart-plus; Sartorius AG, Goettigen, Germany). Dissolved nutrient samples were then stored in 50 ml polyethylene vials and immediately frozen until analysis. Samples for DIC were kept in 12 ml glass vials (Exetainer; Labco, High Wycombe, UK) and preserved with 20 μl of Mercuric (II) chloride and refrigerated until analysis.

Simultaneously, DO concentration and pH were measured using HACH LDO101 (HACH, Colorado USA) optical DO and pH probe. The cores were left to incubate until the O_2 was lowered by 10 – 20 % as described in Dalsgaard et al. (2000). Incubation periods for the sampled sites were between 11 and 24 hours and water samples were collected 4 times over the incubation period. The fluxes of each analyte were calculated based on the change in concentration over time, while taking into consideration the water volume inside the cores and surface area of the sediment (Dalsgaard et al., 2000), and were corrected for any replacement of water.

At the end of the nutrient flux incubation, a 25 μm tip O_2 micro-electrode (Unisense A/S, Aarhus, Denmark) was used to obtain O_2 concentration profiles starting from 20 mm above the sediment surface. The micro-electrode was then lowered into the sediment and at every 0.5 mm depth O_2 saturation and voltage were recorded once the signal settled. The measurements were stopped when O_2 saturation reached 0%. Two incubation cores from each site were used for microprofiling.

All nutrient samples (NH_4^+ , NO_2^- , NO_3^- and PO_4^{3-}) were analysed by the Water Studies Centre (WSC, Monash University) using flow injection analysis (FIA) (Lachat Quikchem 8000 Flow injection Analyser, spectrophotometric detector). All nutrient analyses followed the procedures in Standard Methods for Water and Wastewater (APHA 2005). DIC was analysed based on the Coulometric method using a LI-7000 CO_2/H_2O gas analyzer (LI-COR Biosciences, Lincoln, NE, USA).

Denitrification and dissimilatory nitrate reduction to ammonium (DNRA)

Denitrification incubations were carried out simultaneously with the sediment-water nutrient incubations. Denitrification was measured using the isotope pairing technique described by L. P. Nielsen (1992) whereby the rates were calculated from the accumulation

of labelled N_2 after the addition of labelled $^{15}NO_3^-$ in the cores. At the start of the incubation, a 15 ml water sample was taken prior to the addition of $^{15}NO_3^-$. The water overlying the sediment in the cores were left to mix for ~ 1 minute before another 15 ml water sample was taken to determine the initial concentration of $^{15}N-NO_3^-$. The cores were then capped for incubation (i.e. to allow $^{15}NO_3^-$ to diffuse towards the denitrification zone and reach equilibrium). The measurements of $^{15}NO_3^-$ were carried out as a time series, with incubation times similar to that of the nutrient flux. Each time DO measurements and water samples were taken, one core was sacrificed by adding $ZnCl_2$ to the cores then homogenising them by stirring gently with a glass rod. These cores were left to settle for approximately 1 min before a water sample was taken to calculate N_2 . These samples were preserved in a 12 ml glass vials (Exetainer; Labco, High Wycombe, UK) containing 250 μ l of $ZnCl_2$ 50% w:v until analysis. The isotope pairing technique is now widely used to measure denitrification rates (e.g. K. Nielsen et al., 1995; Sundbäck et al., 2004; F. Wang et al., 2003), however, like most experiment methods there are several assumptions whereby 1) the $^{15}NO_3^-$ must be mixed homogeneously with the $^{14}NO_3^-$ in the sediment, 2) the rate of denitrification does not change as a result of the $^{15}NO_3^-$ addition, 3) the isotope fractionation should be neglected and 4) the $^{15}NO_3^-$ in the overlying water must be able to diffuse into the denitrification zone (Nielsen, 1992). To verify these assumptions, a concentration series experiment was conducted with the addition of 0.2, 0.4 and 0.6 mL of $^{15}NO_3^-$ ($0.05 \text{ mol L}^{-1} \text{ }^{15}NO_3^-$; 98%+, Cambridge Isotope Laboratories) to the water column of nine cores. The values of denitrification were found to be constant at all concentrations. As a result, 0.2 mL of $^{15}NO_3^-$ was used to obtain denitrification rates.

To determine DNRA rates, a sample for $^{15}N-NH_4^+$ was collected at the end of denitrification incubations. The rates were calculated based on a linear increase in the

amount of $^{15}\text{N-NH}_4^+$ produced during the incubation over time as described in Roberts et al. (2012).

Sediment properties

At the end of the nutrient flux incubation, the upper 3 cm of sediment were collected from two cores for sediment porosity, isotopic carbon ($\delta^{13}\text{C}$), isotopic nitrogen ($\delta^{15}\text{N}$), total organic carbon (TOC) content, total nitrogen (TN) content measurements and particle size distribution using a 60 ml cut-off syringe. Sediment porosity was determined from weight loss after oven-drying samples at 105°C to a constant weight. Particle size distribution was determined using laser diffraction, i.e by measuring the distribution pattern of scattered light emitted from the dispersed sediment sample using a Saturn Digisizer 5200 (Micromeritics Instrument Corp., USA). The median diameter (D_{50}) for particle size was determined. Samples for TOC and TN contents and isotopic composition were finely ground prior to analysis, and the sample for TOC analysis was acidified with a dilute HCl solution to dissolve solid carbonates. All samples were analysed at the Water Studies Centre, Monash University on an ANCA GSL2 elemental analyser interfaced to a Hydra 20-22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd., UK). TOC and TN content were expressed as the percentage dry weight of sediment (% d.w.). The precision of TOC and TN content based on replicate analyses (SD for $n=5$) of the same sample was $0.5\ \mu\text{g}$. The analytical precision for stable isotope analysis was $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$ (SD for $n=5$). Stable isotope data were expressed in delta connotation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were calculated from the relative difference between the isotopic ratio within the sample and the isotopic ratio of Vienna Pee Dee Belemnite standard for C and atmospheric N_2 for N.

Macrofauna

Benthic macrofaunal samples were collected from each nutrient flux incubation core at the end of the incubation period. Sediments were sieved through a 1 mm sieve and the material retained on the sieve was preserved with buffered 10 % formalin for at least 48 hours before being rinsed and preserved in 70% ethanol. Macrofauna was sorted from the detritus under a dissecting microscope, identified and enumerated.

Statistical analyses

To identify which environmental predictors best explain each benthic flux, best subsets multiple regression analyses was used. The average value of the replicates for each predictor variable (porosity, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, TOC, TN, C/N molar ratio, bottom water dissolved oxygen (BDO), depth, grain size and faunal abundance) were analysed against each benthic flux. All explanatory variables were included in the initial multiple regression analysis. Multi-collinearity issues were identified using correlation coefficients between the predictor variables and by calculating the variance inflation factor (VIF). VIF >10 indicates multi-collinearity (Quinn & Keough, 2002). $\delta^{13}\text{C}$ was found to be correlated with $\delta^{15}\text{N}$ ($R = 0.80$) while porosity was correlated with TOC ($R = 0.77$), TN ($R = 0.81$) and C/N molar ratio ($R = 0.71$). Grain size was also correlated with BDO ($R = 0.81$) and depth ($R = -0.75$). Therefore, separate models were employed, each one containing one of the collinear predictors until the best subset model was identified using corrected Akaike's information criterion (AICc). Normality of the residuals and homogeneity of variance was checked using the graphical method described by Quinn and Keough (2002) and collinearity of the retained predictor variables in the final models were verified again.

The multivariate analyses of benthic fluxes were performed using principal component ordination (PCO) to determine the relationship of the samples to each other

(ordination) in multidimensional space. PCO was chosen because it is flexible while maintaining its functionality to project points onto axes that minimise residual variation in the space of the resemblance measure chosen (Anderson et al., 2008). Prior to the analysis, homogeneity of each variable was assessed using draftsman plots and all data except for O₂ fluxes required transformation. All flux data were then “normalised” as a standardisation routine in PRIMER v6 (PRIMER-E, United Kingdom), (Clarke & Gorley, 2006). A vector plot was overlaid to determine which fluxes were driving the differences among sites.

A distance-based linear model (DistLM) using stepwise selection, AICc, 9999 n permutations and a significance level of $\alpha = 0.05$ was used to identify which environmental variables best explained the observed variation in benthic fluxes. The predictor variables used in DistLM were identical to those used in the multiple regression. Before any of the predictor variables were added to the model, draftsman plots of each predictor variable were used to detect skewness and check for collinearity. TOC and TN contents were highly correlated ($R > 0.95$). To determine which variable would be excluded, an initial run of the model with all the environmental variables included was undertaken. Based on marginal tests in DistLM, the p -value for TOC content was larger than TN content, therefore the latter variable was retained for possible inclusion in the model. Through addition of each predictor variable, sequential testing examined the statistical contribution of each variable to the explained variation and this was presented as a result of the DistLM. Distance-based redundancy analysis (dbRDA) was used to visualise the results of the DistLM. PCO and DistLM analyses were both performed using the software package PRIMER v6

Results

Water parameters

Bottom water temperatures ranged from 13.6 to 14.5 °C. The temperatures were generally similar throughout the harbour with an average temperature of 14.3 °C (± 0.2 °C), except at CHN and KR1 where temperatures dropped below 14 °C. Salinity readings around central harbour sites and KR1 ranged from 30.4 to 31.1 ppt, while salinity was slightly lower at CHN (29.4 ppt). At WH1 the water was brackish, with salinity reading of 18.2 ppt. Bottom water dissolved oxygen (BDO) concentrations were highest at sites CHN and WH1, whereas sites located around the central harbour had lower DO concentrations, ranging from 1.1 to 2.2 mg/L.

Table 2 Bottom water characteristics at sampling sites in Macquarie Harbour in November 2012.

Site	Depth (m)	Temperature (°C)	Salinity (ppt)	DO (mg/L)
WH1	7	14.3	18.2	5.5
WH2	30	14.5	31.1	1.1
CH1	40	14.3	31.1	2.1
PET3	18	14.1	30.4	1.2
TSC	32	14.3	31.0	2.2
CHE	18	14.3	30.6	1.8
CH2	35	14.3	31.0	1.9
CHN	13	13.6	29.4	3.5
KR1	33	13.9	31.1	2.5

Sediment description

The elemental composition and stable isotopic results for each sampling site are shown in Figure 4. Carbon signatures ($\delta^{13}\text{C}$) were similar across sites, ranging from -27.04 ± 0.01 to 28.22 ± 0.86 ‰. Nitrogen signature ($\delta^{15}\text{N}$) was lowest in the upper estuary (WH1) at 1.6 ± 0.01 ‰ and increased to 4.6 ± 0.16 ‰ in the lower estuary. There appeared to be a

similar pattern in the distribution of total organic carbon (TOC) and total nitrogen (TN) content throughout the harbour. TOC and TN content were lowest at the site closest to the mouth (CHN) whereas sites located in the middle of the harbour generally had higher TOC and TN content. Sediment in the upper estuary had C/N molar ratios (23.69 ± 7.53) similar to those in the mid estuary ($20.88 \pm 0.23 - 24.08 \pm 0.86$), whereas the C/N molar ratio was low at CHN (15.93 ± 0.23) compared to other sites.

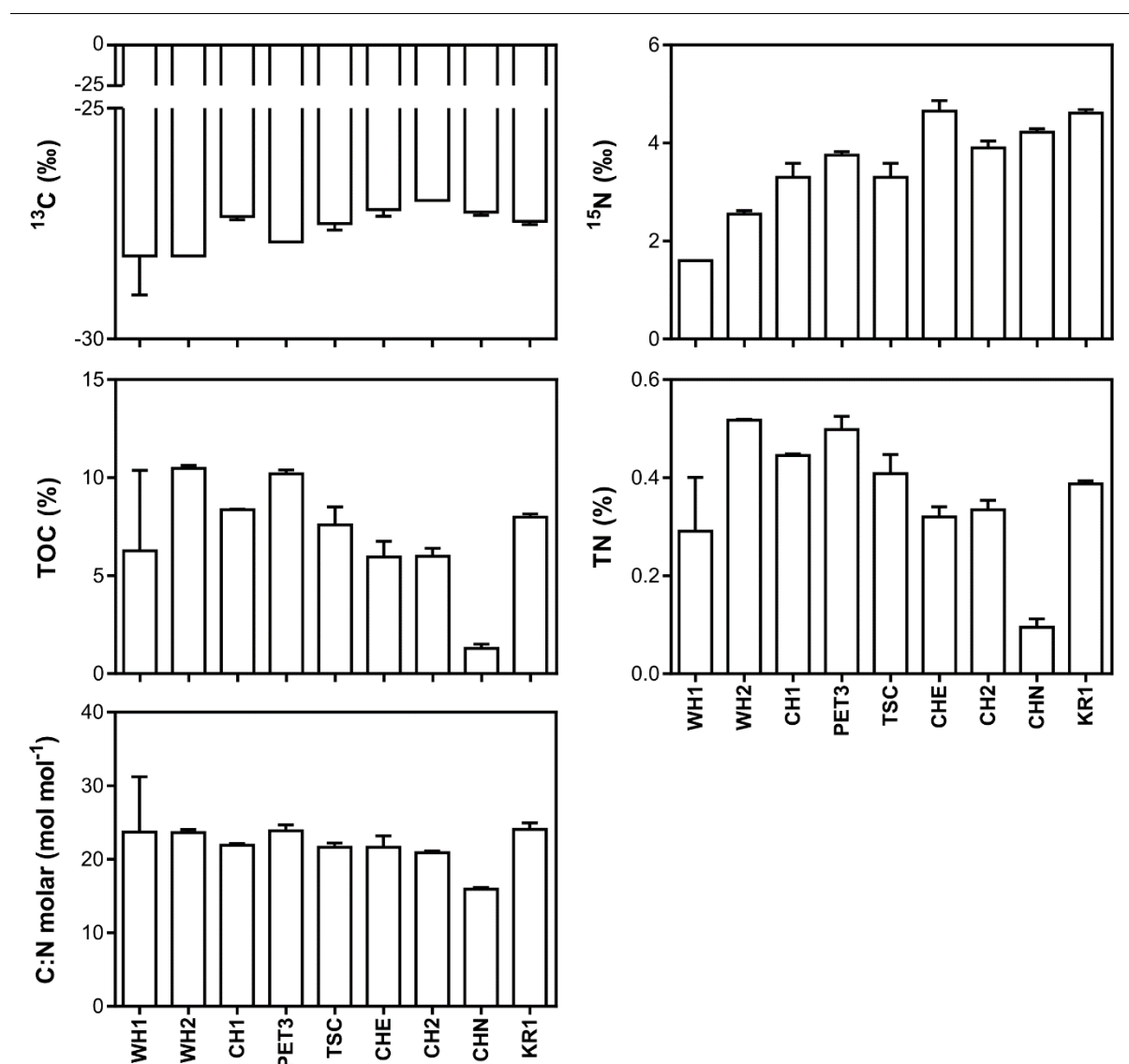


Figure 4 $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C/N molar ratio, TOC and TN content from the top 3 cm of sediments taken in Macquarie Harbour. Error bar indicates standard deviation of the mean of 2 samples taken from two separate cores.

Sediment porosities were generally similar across sites, with the exception of WH1 and CHN as shown in Table 3. Sediment porosity was low at the southernmost site, WH1 (0.58 ± 0.00) increasing towards the central harbour with values ranging from 0.71 ± 0.05 to 0.82 ± 0.03 before decreasing again in the northern reaches of the harbour at CHN (0.37 ± 0.05). Highest porosity was at KR1 with 0.84 ± 0.02 . A similar pattern was observed for median grain size. Median grain size value was higher at WH1 ($44.40 \pm 18.16 \mu\text{m}$) and CHN ($18.63 \pm 10.28 \mu\text{m}$) compared to sites WH2, CH1, PET3, TSC, CHE and CH2 in mid estuary (ranging from 6.74 ± 0.44 to 11.54 ± 0.12). Oxygen penetration depth (OPD) was highest at WH1 and CH2 at $3.0 \pm 1.0 \text{ mm}$ while other sites ranged from $1.0 \pm 0.5 \text{ mm}$ to $2.5 \pm 10.5 \text{ mm}$.

Table 3 Average porosity, median grain size and oxygen penetration depth (OPD). Error (\pm) indicates standard deviation of the mean of 2 samples taken from two separate cores.

Site	Porosity	Median grain size (μm)	OPD (mm)
WH1	0.58 ± 0.00	44.40 ± 18.16	3.0 ± 1.0
WH2	0.76 ± 0.08	10.48 ± 0.34	1.5 ± 0.0
CH1	0.81 ± 0.03	7.69 ± 0.98	2.0 ± 0.0
PET3	0.81 ± 0.02	11.54 ± 0.12	2.5 ± 0.5
TSC	0.82 ± 0.03	9.47 ± 0.95	2.5 ± 0.0
CHE	0.79 ± 0.00	6.85 ± 0.05	2.0 ± 1.0
CH2	0.71 ± 0.05	6.74 ± 0.44	3.0 ± 1.0
CHN	0.37 ± 0.05	18.63 ± 10.28	No data
KR1	0.84 ± 0.02	8.68 ± 1.05	1.0 ± 0.5

Benthic flux rates

The calculated fluxes across the sediment-water interface at all sites are shown in Figure 5. Oxygen (O_2) flux rates ranged from -2.98 ± 1.57 to $-17.22 \pm 1.10 \text{ mmol m}^{-2} \text{ d}^{-1}$. Most sites in the middle of the harbour (WH2, CH1, PET3, TSC, CHE, CH2) showed lower O_2 consumption rates, notably WH2 ($-2.98 \pm 1.57 \text{ mmol m}^{-2} \text{ d}^{-1}$), CHE ($-4.09 \pm 2.76 \text{ mmol}$

$\text{m}^{-2} \text{d}^{-1}$) and CH1 ($-4.25 \pm 1.87 \text{ mmol m}^{-2} \text{d}^{-1}$) compared with WH1 ($-17.22 \pm 1.10 \text{ mmol m}^{-2} \text{d}^{-1}$) in the upper reaches of the harbour and CHN ($-13.84 \pm 3.67 \text{ mmol m}^{-2} \text{d}^{-1}$) closest to the mouth of the harbour. The highest dissolved inorganic carbon (DIC) efflux was at KR1 ($40.24 \pm 7.91 \text{ mmol m}^{-2} \text{d}^{-1}$), with a flux rate three times higher compared to the other sites. Aside from KR1, DIC efflux rates ranged from 3.78 ± 1.3 to $13.16 \pm 7.05 \text{ mmol m}^{-2} \text{d}^{-1}$. Uptake of PO_4^{3-} was high at CHN ($-0.035 \pm 0.02 \text{ mmol m}^{-2} \text{d}^{-1}$), whereas the uptake rates of PO_4^{3-} at the other sites ranged from -0.002 ± 0.01 to $-0.017 \pm 0.00 \text{ mmol m}^{-2} \text{d}^{-1}$.

There was a large efflux of ammonium (NH_4^+) at KR1 ($2.01 \pm 0.37 \text{ mmol m}^{-2} \text{d}^{-1}$); five times higher compared to the other sites. More generally, the efflux of NH_4^+ ranged from 0.09 ± 0.05 to $0.35 \pm 0.13 \text{ mmol m}^{-2} \text{d}^{-1}$ except at CHN where there was an uptake of $-0.17 \pm 0.10 \text{ mmol m}^{-2} \text{d}^{-1}$. There were no changes in nitrite (NO_2^-) concentrations at sites WH1, WH2, CH1 and TSC. At the other five sites where NO_2^- concentrations were detected, KR1 had the highest efflux of NO_2^- ($0.04 \pm 0.01 \text{ mmol m}^{-2} \text{d}^{-1}$) followed by CHE and PET3 whereas CH2 had the highest uptake at $0.02 \pm 0.00 \text{ mmol m}^{-2} \text{d}^{-1}$ followed by CHN. For nitrate (NO_3^-) fluxes, sediment uptake occurred at all sites except at WH1, where the efflux rate was $0.030 \pm 0.08 \text{ mmol m}^{-2} \text{d}^{-1}$. Highest NO_3^- uptake rate was at KR1 ($-0.409 \pm 0.02 \text{ mmol m}^{-2} \text{d}^{-1}$) while the lowest was at CHN ($-0.0043 \pm 0.09 \text{ mmol m}^{-2} \text{d}^{-1}$).

Total denitrification rates ranged from 0.055 to $0.215 \text{ mmol m}^{-2} \text{d}^{-1}$. From the total denitrification rates, uncoupled nitrification-denitrification (D_w) was lowest at KR1 ($0.007 \text{ mmol m}^{-2} \text{d}^{-1}$) and highest at CHE ($0.084 \text{ mmol m}^{-2} \text{d}^{-1}$), whereas coupled nitrification-denitrification (D_n) ranged from 0.019 to $0.157 \text{ mmol m}^{-2} \text{d}^{-1}$. The rate of dissimilatory nitrate reduction to ammonium (DNRA) varied among sites, but the lowest rate was recorded at CH2 and the highest at KR1.

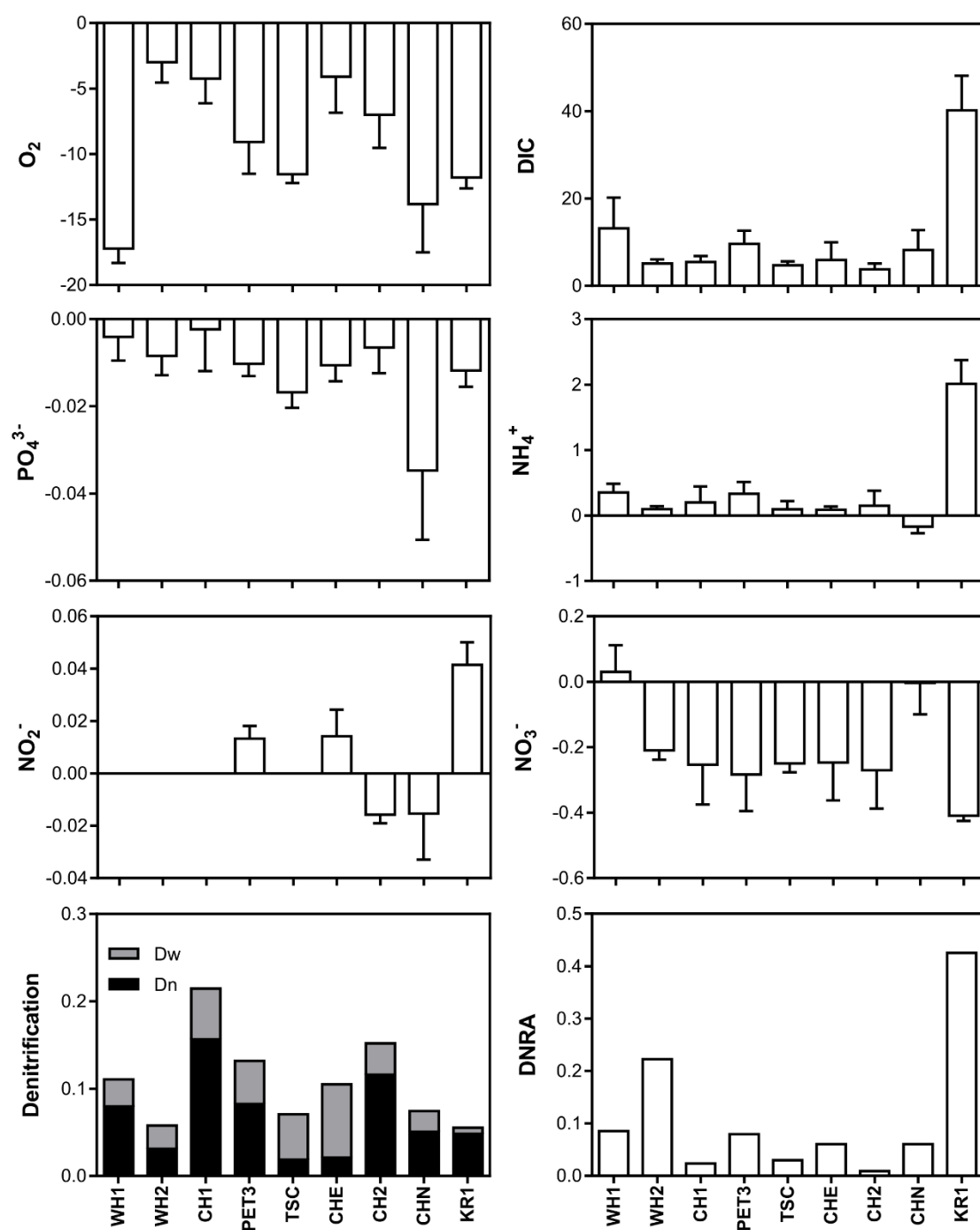


Figure 5 Flux rates of O₂, DIC, PO₄³⁻, NH₄⁺, NO₂⁻, NO₃⁻ and denitrification during incubations of sediments taken from the control sites. All data are presented in mmol m⁻² d⁻¹. Error bars indicate standard deviation of the mean (n= 3 or 4). Total denitrification rates are the sum of uncoupled (Dw) and coupled nitrification-denitrification (Dn) rates.

Macrofauna

Macrofauna data are shown in Table 4. Twenty different taxonomic groups were identified, although the samples were dominated by annelids. The site located at the lower

reaches of the harbour (CHN) had the highest diversity of macrofauna, with identified individuals coming from 9 different taxonomic groups, while CH2 had the highest abundance with 22 individuals.

Table 4 Summary of benthic fauna identified. The data represents the total number of individuals collected from 4 sediment cores of each sampling sites. The average and standard deviation of each site are also shown.

Family	WH1	WH2	CH1	PET3	TSC	CHE	CH2	CHN	KR1
Phoxocephalidae	0	0	1	0	0	0	5	0	0
Cirolanidae	0	1	0	0	0	0	0	1	0
Anthuridae	0	0	0	0	0	0	0	1	0
Nebaliidae	0	0	0	1	0	0	0	0	0
Ostracoda (Class)	0	0	0	0	1	0	0	2	0
Chiridotidae	0	0	0	0	0	0	0	1	0
Hydrobiidae	2	0	0	0	0	0	0	0	0
Lucinidae	0	0	0	0	0	0	0	1	0
Ungulinidae	0	0	0	0	0	1	0	0	0
Thyasiridae	0	0	0	0	1	0	0	1	0
Nemertea (Phylum)	0	0	0	0	0	0	1	0	0
Flabelligeridae	0	1	0	0	0	0	0	0	0
Goniadidae	1	0	0	0	0	0	0	0	0
Sabellidae	0	0	0	0	0	0	0	3	0
Maldanidae	7	0	0	0	0	0	0	0	0
Orbiniidae	6	0	0	0	0	1	1	0	0
Spionidae	2	0	0	0	7	0	0	0	0
Terebellidae	0	0	1	2	3	0	15	6	0
Ampharetidae	0	1	0	0	0	0	0	0	0
Gobiidae	0	0	0	0	0	0	0	1	0
Total	18	3	2	3	12	2	22	17	0
Average	4.5	0.8	0.5	0.8	3.0	0.5	5.5	4.3	0
Standard dev.	3.7	0.1	0.1	0.5	1.2	0.6	2.5	2.6	0

Environmental drivers of benthic fluxes

The subset models which best explained each of the benthic fluxes are presented in Table 5. The set of environmental variables could explain more than 45% of each benthic flux rate, most notably BDO (70%). Respiration (measured as O₂ uptake) strongly increased with the increase in BDO concentration, $\delta^{15}\text{N}$ and TOC content but decreased with increasing depth. Respiration (measured as DIC release) also increased with increasing BDO concentrations, $\delta^{15}\text{N}$ and TOC content but decreased with faunal abundance. Increases in PO₄³⁻ release were associated with higher BDO concentrations and sediment TN content. The release of NH₄⁺ from the sediment was also positively correlated with increasing BDO, TOC and $\delta^{15}\text{N}$ but negatively correlated with total faunal abundance. Meanwhile, NO₂⁻ flux rate increased with increasing BDO concentration, porosity and depth. NO₃⁻ flux rates were positively correlated with increasing in median grain size and negatively correlated with the C/N molar ratio of sediment organic matter.

Table 5 Results of multiple linear regression model (using stepwise procedure) determining the environmental driver of each of the key benthic flux in Macquarie Harbour. Metric coefficient was also presented. Where the metric coefficient is not presented for a given predictor variable, this indicates that the particular variable was not retained in the model.

Flux	Adj. R ²	p	F	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	TOC	TN	C:N	Porosity	BDO	Grain Size	Fauna	Depth
O ₂	0.70	<0.001	21.19	a	3.72 ^a	1.02 ^b	b	b	b	4.33 ^c	c		-0.16
NH ₄ ⁺	0.66	<0.001	17.62	a	0.69 ^a	0.30 ^b	b	b	b	0.75 ^c	c	-0.16	
PO ₄ ³⁻	0.52	<0.001	19.41			b	0.09 ^b	b	b	0.01 ^c	c		
NO ₂ ⁻	0.45	<0.001	10.32			b	b	b	-0.01 ^b	0.01 ^c	c		0.001
NO ₃ ⁻	0.64	<0.001	31.25			b	b	-0.03	b	c	0.01 ^c		
DIC	0.59	<0.001	12.96	a	10.80 ^a	4.14 ^b	b	b	b	13.10 ^c	c	-3.07	

a, b, c Indicates that the variables are correlated. Only one variable from each group of collinear variables was used in the model (selection explained in methods).

Spatial variability and the influence of environmental drivers on the suite of benthic fluxes

PCO plot shows that the first two PCO axes explained 61.31% of the total variation in the flux data with PCO1 explaining 37.2% of the total variance (Figure 6). The first two

axes of the dbRDA plot of predictor variables showed 65.69% variability in the fitted model and 57.62% of total variation, which indicated that most of the relevant patterns in the model were captured. The PCO plot showed that the fluxes at KR1, CHN differed from all other sites, and that this was mostly driven by NO_3^- , NO_2^- and NH_4^+ . DNRA and DIC seemed to have driven the separation between KR1 while total denitrification, Dn, and Dw compel all the other sites (Table 6). The result for the best DistLM showed that all of the predictor variables included in the model significantly driving the benthic flux rates except for porosity (Figure 7, Table 7). C/N molar ratio, TN, depth, BDO, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, median grain size and fauna together explained 87.7% of the variation in the fluxes. The vector plot for DistLM showed that the driver of the fluxes at CHN was C/N molar ratio, BDO and depth for KR1 and TN for all other sites. The dbRDA plot showed similar patterns to the benthic fluxes PCO plot which indicates that the DistLM captured the overall patterns of the variability.

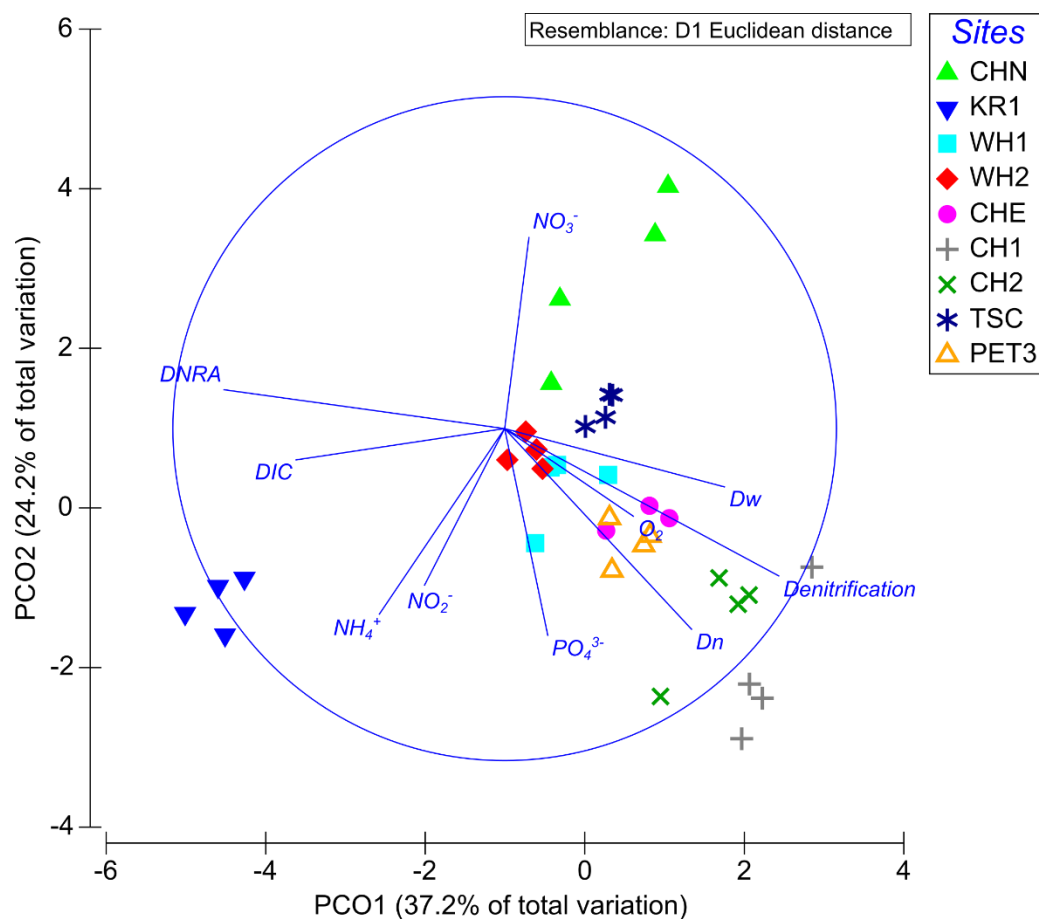


Figure 6 Principal component analysis (PCO) plot showing the non-metric multivariate similarity among replicated benthic fluxes of nine sites in Macquarie Harbour.

Table 6 The eigenvalue and eigenvector of the fluxes on the first five principal component axes.

	PCO1	PCO2	PCO3	PCO4	PCO5
Eigenvalue	126.32	82.142	51.461	23.308	22.057
Individual %	37.15	24.16	15.14	6.86	6.49
Eigenvector					
Denitrification	-0.828	-0.444	-0.397	-0.044	-0.060
Dn	-0.565	-0.606	-0.607	-0.398	-0.230
DNRA	0.848	0.118	0.085	0.163	-0.392
Dw	-0.664	-0.176	0.288	0.436	0.136
PO_4^{3-}	-0.130	-0.624	-0.097	0.315	-0.318
DIC	0.631	-0.094	-0.326	0.099	-0.106
O_2	-0.389	-0.265	0.602	-0.222	-0.346
NO_3^-	-0.073	0.578	-0.472	0.220	-0.369
NH_4^+	0.379	-0.560	-0.144	0.253	-0.054
NO_2^-	0.242	-0.473	0.372	-0.031	0.465

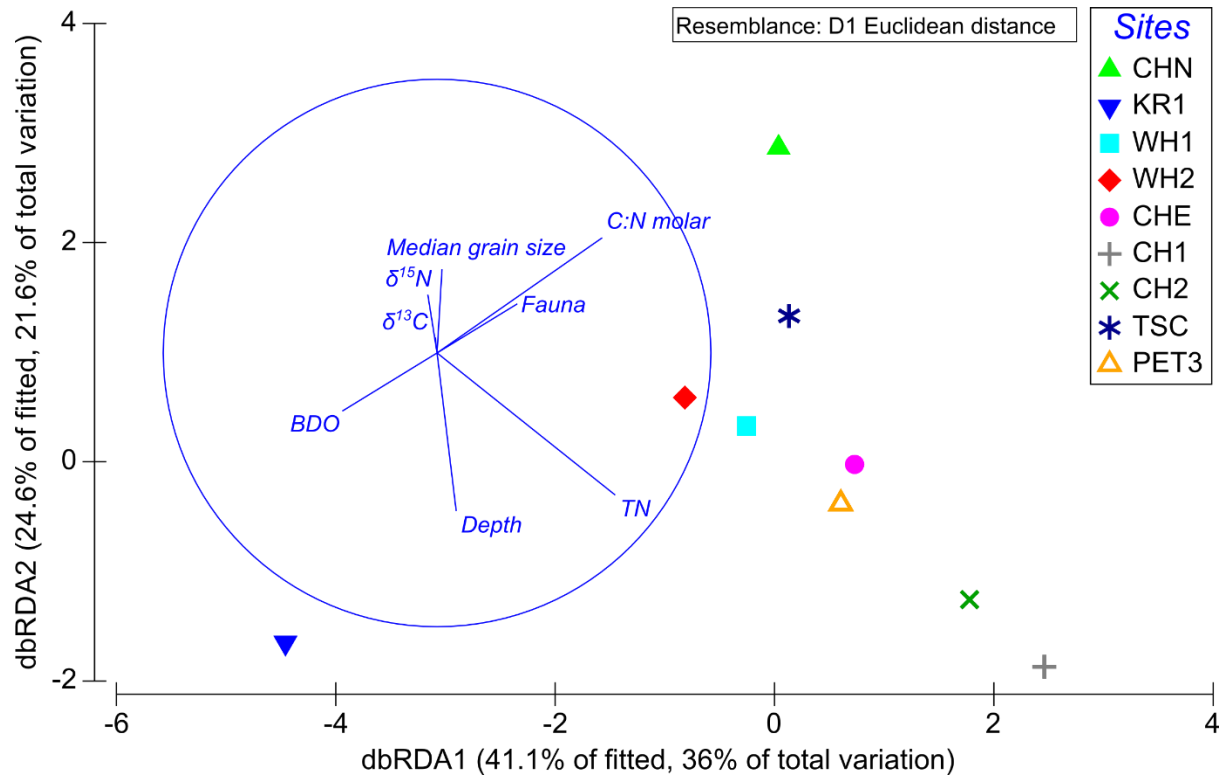


Figure 7 dbRDA plot of the DistLM based on environmental drivers fitted to the variation of the benthic fluxes from nine sites in Macquarie Harbour.

Table 7 DistLM on the relation of environmental variables to the benthic fluxes from nine sites. The variables is explained using stepwise sequential tests, AICc selection criterion, $n = 35$ and 9999 permutations.

Variable	AICc	SS(trace)	Pseudo- F	P	Percent variation explained	Cumulative variation explained
C:N molar	76.53	64.95	7.79	<0.001	19.10	19.10
TN	69.48	65.07	9.92	<0.001	19.14	38.24
Depth	64.66	39.94	7.28	<0.001	11.75	49.99
BDO	56.95	43.87	10.43	<0.001	12.90	62.89
$\delta^{13}\text{C}$	53.23	21.84	6.07	<0.001	6.42	69.32
$\delta^{15}\text{N}$	46.39	25.90	9.25	<0.001	7.62	76.93
Median grain size	36.75	24.37	12.18	<0.001	7.17	84.10
Average fauna abundance	31.38	12.29	7.65	<0.001	3.61	87.72

Axis	% explained variation out of fitted model		% explained variation out of total variation	
	Individual	Cumulative	Individual	Cumulative
1	41.05	41.05	36.01	36.01
2	24.64	65.69	21.61	57.62

3	16.31	82.00	14.31	71.93
4	6.52	88.53	5.72	77.35
5	5.96	94.49	5.23	82.88
6	3.85	98.34	3.38	86.26
7	1.66	100	1.46	87.71

Excluding KR1, the PCO and DistLM analyses were repeated to further investigate the spatial variability and environmental drivers influencing overall flux rates. The PCO plot shows that the first two PCO axes explained 55.32% of the total variance with 36.13% being explained by the first PCO axis and 19.19% by the second axis (Figure 8). Total denitrification dominated the first PCO axis (Table 8). DNRA and NO_3^- flux rates separate CHN from the other sites. Meanwhile, DIC flux rates were different at WH1 compared to the other rates from all other sites. Dw, NO_2^- and denitrification drove the separation between CH1 and CH2 and all the other sites. The best DistLM results comprised a mix of median grain size, C/N molar ratio, BDO concentration, $\delta^{13}\text{C}$, TN content, porosity and faunal abundance (Figure 9, Table 9). Median grain size is the most likely environmental variable driving the flux rates for WH1 and PET3. The differences in flux rates at WH2, CHE and TSC seemed to be driven by C/N molar ratio and as for CH1 and CH2 its TN.

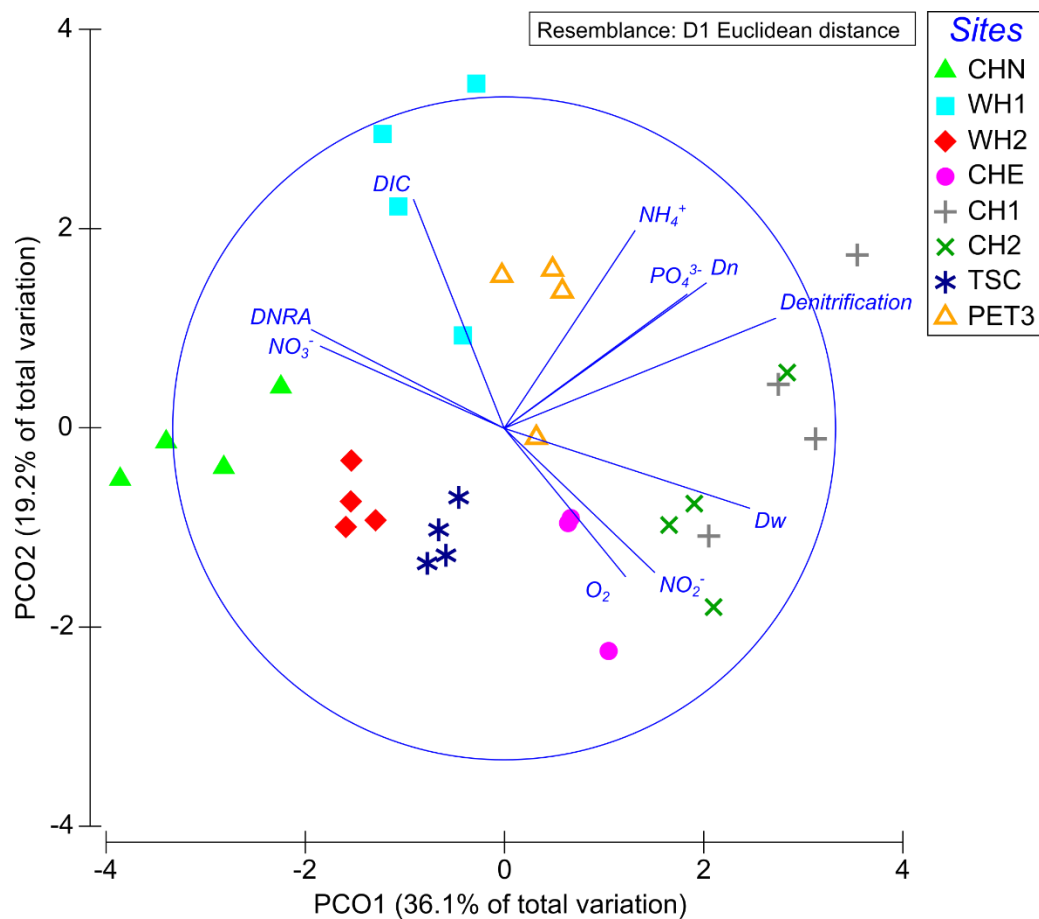


Figure 8 PCO plot showing the non-metric multivariate similarity among replicated benthic fluxes of eight sites (excluding KR1) in Macquarie Harbour.

Table 8 The eigenvalue and eigenvector of the fluxes taken from only eight sites on the first five principal component axes.

	PCO1	PCO2	PCO3	PCO4	PCO5
Eigenvalue	108.39	57.578	44.188	26.559	23.876
Individual %	36.13	19.19	14.73	8.85	7.96
Eigenvector					
O ₂	-0.367	0.448	-0.298	-0.532	0.352
NH ₄ ⁺	-0.396	-0.598	-0.347	0.075	-0.068
PO ₄ ³⁻	-0.553	-0.405	-0.183	0.113	0.397
NO ₂ ⁻	-0.455	0.435	-0.180	0.569	0.289
NO ₃ ⁻	0.556	-0.248	0.273	0.230	0.356
DIC	0.274	-0.692	-0.190	0.015	-0.331
Dw	-0.742	0.242	-0.209	0.052	-0.458
Dn	-0.611	-0.439	0.567	-0.256	0.230
Denitrification	-0.820	-0.333	0.521	-0.063	-0.035
DNRA	0.583	-0.298	-0.596	-0.163	0.176

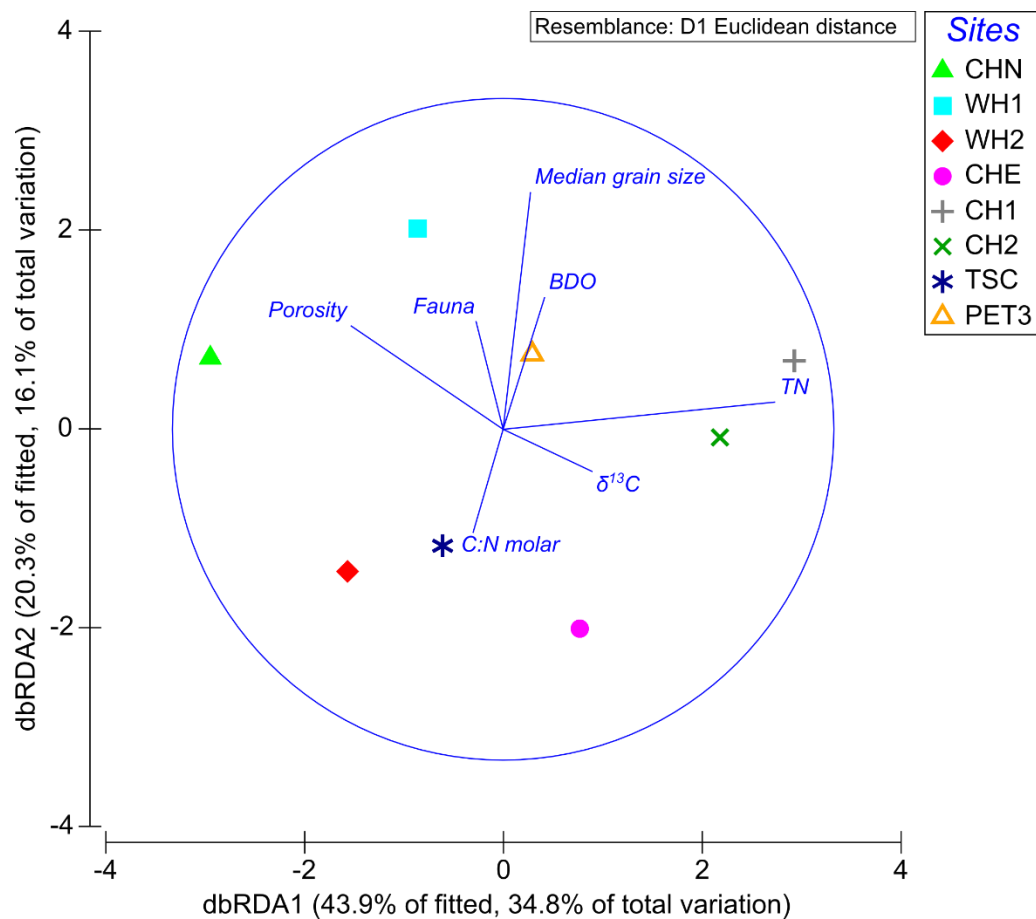


Figure 9 dbRDA plot of the DistLM based on environmental drivers fitted to the variation of the benthic fluxes from eight sites (excluding KR1).

Table 9 DistLM on the relation of environmental variables to the benthic fluxes from eight sites. The variables is explained using stepwise sequential tests.

Variable	AICc	SS(trace)	Pseudo- <i>F</i>	<i>P</i>	Percent variation explained	Cumulative variation explained
Median grain size	65.23	79.60	10.47	<0.001	26.54	26.54
C:N molar	62.35	34.91	5.27	<0.001	11.64	38.17
BDO	59.21	31.60	5.54	<0.001	10.53	48.70
$\delta^{13}\text{N}$	57.35	21.76	4.28	<0.001	7.25	55.96
TN	55.99	17.68	3.85	<0.001	5.89	61.85
Porosity	48.36	34.20	10.23	<0.001	11.40	73.25
Average fauna abundance	44.02	18.29	6.79	<0.001	6.10	79.34

Axis	% explained variation out of fitted model		% explained variation out of total variation	
	Individual	Cumulative	Individual	Cumulative
1	43.90	43.90	34.84	34.84
2	20.31	64.22	16.12	50.95
3	14.17	78.39	11.25	62.20
4	9.58	87.97	7.60	69.80
5	8.52	96.50	6.76	76.57
6	2.97	99.47	2.36	78.92
7	0.53	100	0.42	79.34

Discussion

This study identified significant spatial variability in nutrient flux and benthic respiration rates in Macquarie Harbour. Benthic fluxes often vary spatially in estuaries due to the inherent hydrodynamic, biological and chemical complexity of these systems. When seeking to understand the influence of any additional anthropogenic inputs to a system (such as marine farming) it is important to understand the natural variability in order to appropriately differentiate and manage any resultant impacts. The present study is the first reported measure of benthic respiration and nutrient fluxes at the sediment-water interface in Macquarie Harbour. The results clearly outline how the differences in the background environmental conditions affect mineralisation processes and thereby provides information that will be critical for effective ecosystem management in the harbour.

Sediment characteristics

In this study, the top 3 cm of sediment was sampled and homogenised for sediment analysis. This depth was chosen as it is likely to integrate effects on sediment characteristics in the harbour over the last ~ 5 to 10 years. The sediments at the shallower locations in the upper and lower parts of the harbour had a larger median grain size, probably due to high energy riverine and marine forcings. In the deeper central region the sediments were

predominantly smaller in median grain size, most likely as a result of low turbulence and physical forces allowing a greater level of settlement (Hart, 1995) and the breakdown of sediment grains in the water column during sedimentation (Middelburg et al., 1993).

Teasdale et al. (2003) suggested that organic matter content of Macquarie Harbour sediments increased from north to south. Untangling such a pattern in this study is difficult because of differences in depth and the proximity to other sources of organic matter (e.g. King River in the lower reaches of the harbour). However, given that the sites in the central harbour are of similar depth (sites WH2, CH1, TSC and CH2) there does appear to be a north to south pattern with decreasing levels of total organic carbon (TOC) and total nitrogen (TN) from WH2 which is at the upper reaches of the harbour towards CH2 at the lower part of the estuary.

The isotope signatures and C/N molar ratio indicate that the organic matter in the sediments sampled were mainly terrestrial sourced, composed of vascular plants which naturally have a low $\delta^{13}\text{C}$ signature and high C/N (Bianchi, 2006). The lower $\delta^{13}\text{C}$ signatures were found at sites closer to the Gordon River (WH1 and WH2) and the values increased across the harbour towards the lower reaches of the estuary. This pattern reflects the contribution of marine or estuarine phytoplankton which are $\delta^{13}\text{C}$ enriched (-18 to -24) compared to the more depleted $\delta^{13}\text{C}$ of terrigenous origin of organic matter (-26 to -30) and freshwater phytoplankton (-24 to -30) at the lower reaches of the harbour (Bianchi, 2006). $\delta^{15}\text{N}$ also showed a gradient from north to south, with higher values close to the harbour entrance and decreasing towards the upper reaches of the harbour. The spatial variation in $\delta^{15}\text{N}$ may have been caused by marine organic matter and organisms, which often have higher $\delta^{15}\text{N}$ values, brought in through tidal mixing (Finlay & Kendall, 2005). $\delta^{15}\text{N}$ are also found to be depleted in terrestrial organic matter (Cloern et al., 2002) which may be attributed to intensive transformation of N in freshwater sediments (Finlay & Kendall, 2005;

Ostrom et al., 2002). C/N molar ratios of Macquarie Harbour sediments were also within the range for vascular plants (20-500) except at the mouth of the harbour (CHN) where the value was lower indicating a mix of vascular plants and plankton (Hedges et al., 1988). The isotope signatures showed a gradient similar to that commonly observed in estuaries such as the Huon estuary in Tasmania (CSIRO, 2000), the Schelde estuary (Middelburg & Nieuwenhuize, 1998), and San Francisco bay (Cloern et al., 2002) with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values increasing from the upper to the lower estuary.

Sediment respiration

Higher rates of oxygen consumption were measured in sediments taken from the sites closest to the riverine and marine boundaries (i.e. CHN, KR1 and WH1). Meanwhile, the sites located around central harbour (especially WH2, CH1, CHE and CH2) all had lower oxygen consumption rates. These sites also had the lowest bottom water dissolved oxygen concentrations, which may have implications for oxygen availability and the resultant ability to instigate microbial aerobic respiration during mineralisation of organic matter and oxidation reactions (Middelburg & Levin, 2009; Middelburg et al., 1993). Murrell and Lehrter (2011) have previously identified that sediment oxygen consumption is dependent on the availability of oxygen in the overlying water; with sediment oxygen consumption being low when bottom water oxygen is low but increasing rapidly when the overlying water is re-oxygenated. It is important to note that the oxygen consumption is not only a function of respiration but will also occur as a result of chemical re-oxidation of anaerobic metabolites such as iron (II) and hydrogen sulfide produced during anaerobic respiration during low oxygen concentration periods. Studies have also shown that oxygen consumption can be inversely proportional to the depth of the overlying water column (Hopkinson & Smith, 2004) and that the deposition of organic matter to the sediment

depends on the depth of the overlying water (Middelburg et al., 1993). This suggests that at greater depth, organic matter is more likely to be mineralised in the water column before reaching the sediment, leaving more recalcitrant components which are more difficult to decompose, which might also explain the low oxygen consumption rates.

Although oxygen consumption is widely proposed as the most effective way to represent organic matter mineralisation (Glud, 2008; Lansard et al., 2008), in this study, given evidence of anaerobic mineralisation in Macquarie harbour sediments, the flux of dissolved inorganic carbon (DIC) gives a more complete picture of total sediment mineralisation. In aerobic environments, the ratio of DIC production against oxygen consumption, referred to as the respiratory quotient (RQ), is generally fairly close to 1 (Hopkinson & Smith, 2004). In this study, DIC/O₂ ratios at the site near the marine boundary (WH1), several of the mid-estuary sites CH1, CHE and King River site (KR1) were well above 1 suggesting that some of the respiration was via anaerobic pathways. A long residence time and associated low dissolved oxygen concentration are natural features of Macquarie Harbour bottom waters (Cresswell et al., 1989; DPIPWE et al., 2011; Koehnken, 1996), and this could result in an adaptation or shift in bacterial communities that use other electron acceptors such as nitrate, manganese oxide, iron oxide and sulfate during decomposition of organic matter (Middelburg & Levin, 2009). The bottom water dissolved oxygen concentrations in the central harbour were either hypoxic (i.e. < 2.0 mg/L) (Middelburg & Levin, 2009) or close to hypoxic at CH1 and TSC. Therefore the likelihood of anaerobic respiration occurring at these sites is high. The three-fold increase in DIC production compared to oxygen consumption at KR1 may indicate that anaerobic respiration is prevalent at this site. It is important to note that although bottom water oxygen concentrations are naturally low in the harbour, concentrations would appear to have dropped even further in recent years compared to historical records (MHDOWG, 2014).

Dissolved oxygen concentrations recorded between 1994 and 2008 rarely fell below 2.5 mg/L. However since 2009 the concentration had been on a decline. Data collected from 2011 onwards together with this study suggest that the bottom water dissolved oxygen levels are regularly hypoxic (< 2.0 mg/L) at sites greater than 15 m. The drop in dissolved oxygen could be attributed to a number of factors including natural changes in the system (factors such as variability in river flow, weather and tidal conditions) and anthropogenic changes (i.e. the increase in, salmon aquaculture in the harbour) (MHDOWG, 2014). The current data does not allow for attribution of cause.

Inorganic fluxes and transformation of nitrogen

The combination of bottom water dissolved oxygen concentration, quantity (measured as TOC, TN) and quality (inferred from C/N molar ratio, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of organic matter and abundance of benthic fauna were significantly correlated to the rates of ammonium regeneration in Macquarie Harbour. However, bottom water dissolved oxygen concentration was the factor most clearly correlated with sediment ammonium release.

Ammonium was released from most of the sediment samples collected within Macquarie Harbour. Efflux of ammonium suggests that the process of denitrification is compromised in these sediments and a lower capacity for the conversion of ammonium to nitrate via nitrification in the sediments is the most likely explanation of this finding. There are a number of factors that could drive this response and the lower capacity for nitrification in Macquarie Harbour sediments, these include the low bottom water oxygen concentrations, low oxygen penetration depths or presence of sulfide (Caffrey et al., 1993; Joye & Anderson, 2008; Kemp et al., 1990; Koike & Sørensen, 1988; Rysgaard et al., 1996). Low bottom water oxygen concentration and penetration depth would also increase the potential for anaerobic mineralisation where microbes would reduce nitrate to produce ammonium

through DNRA (Thamdrup, 2012). In the current study, the abundance of benthic fauna was also found to influence the efflux of ammonium. Burrowing activities affect the depth of oxygen penetration by increasing the size of the oxic surface area (Kristensen, 1988; Kristensen & Holmer, 2001), thereby increasing mineralisation of organic matter and the capacity for coupled nitrification-denitrification in the sediments. This could also potentially be the explanation for the high efflux of ammonium at KR1 despite the higher bottom water oxygen concentration. The effect of low oxygen penetration depth and the absence of benthic fauna were probably key factors in the high efflux of ammonium at this site. However, the fact that ammonium efflux was observed at most sites suggests that these sediments may be inherently functioning close to their assimilatory capacity with respect to denitrification and organic mineralisation and that in this particular system the water column may play a key role in the denitrification process.

Because of the reduced capacity for sediment nitrification due to low oxygen availability, both denitrification and DNRA processes in Macquarie Harbour may have relied on nitrate sourced from the water column. Denitrification which requires sources of nitrate from the overlying water column and/or through nitrification of ammonium in the sediment (Jenkins & Kemp, 1984) is therefore limited by the amount of nitrate (Christensen et al., 1990). In general within Macquarie Harbour 54 to 87% of nitrogen removal was from coupled nitrification-denitrification, except at sites CHE and TSC where only 20% and 26% of the denitrification were coupled. Coupling of the nitrification-denitrification processes can be stimulated indirectly by higher concentration of oxygen and ammonium (Jäntti & Hietanen, 2012). Despite the low oxygen penetration depth at KR1, coupled nitrification-denitrification was found to account for 87% of the total denitrification rate, which also means that a large proportion of the nitrate taken from the water column was for DNRA. Nonetheless, the fact that there was between 13 to 80% uncoupled denitrification implies

that nitrate in the overlying water is an important source for denitrification in the harbour. As coupled nitrification-denitrification is dependent on nitrate through sediment nitrification, uncoupled denitrification will be directly proportional to the nitrate concentration in the overlying water column, where denitrifying bacteria are reported to rapidly respond to the increase in nitrate concentration (Cornwell et al., 1999; Kana et al., 1998).. Several studies have reported the importance of water column nitrate for denitrification in estuarine sediments (Lohse et al 1993; Nielsen et al 1995). The fact that total ammonia nitrogen in the harbour has remained stable despite sediment efflux of ammonium into the water column (see MHDOWG, 2014) also supports the assertion that the release of ammonium from the sediment probably goes through nitrification in the water column, with the resultant nitrate then being available for denitrification.

Previous studies by Carpenter et al. (1991) and Teasdale et al. (2003) have reported the presence of sulfate-reducing bacteria within the harbour, with a strong smell of sulfide in sediment collected from the harbour and high acid volatile sulfide (AVS) values reported especially around King River. This is important as the presence of sulfide can influence N transformation through inhibition of nitrification and denitrification while encouraging DNRA (An & Gardner, 2002). High concentration of nitrate in the water column and sulfide in sediments may favour the coupling of DNRA and sulfide oxidation by sulfate-reducing bacteria (An & Gardner, 2002; Sayama, 2001; Thamdrup, 2012). If this is the case, it is possible that the sulfide in Macquarie Harbour could influence the rate of denitrification and DNRA, increasing the likelihood of nitrate reduction and further decreasing the denitrification efficiency.

Phosphate flux

There was a general influx of phosphate in sediments of Macquarie Harbour. Sedimentary phosphorus burial is the main phosphate removal pathway, hence, it is likely to play a crucial role in regulating phosphorus availability in the water column and primary productivity (Burdige, 2006). The burial of phosphorus is mainly controlled by oxygen concentration and iron redox chemistry on the surface of the sediment (House, 2003; Kraal et al., 2015; Middelburg & Levin, 2009). The influx of phosphate to sediments suggests that diffusing phosphate from organic matter mineralisation or phosphate from the overlying water column is most likely adsorbed by surficial iron or manganese oxides (Maher & Devries, 1994; Nedwell et al., 1999). The adsorption of phosphate could also be linked to oxygen concentration in the sediments. This may be the circumstance that cause a large influx of phosphorus in sediments taken from CHN. One point of difference at this site is that the sediments were coarser than at other locations. Therefore it may be hypothesised that because of the nature of the sediment at this site, oxygen would be more rapidly transported deeper into the sediment and that this could promote the binding of phosphate through subsurface manganese and iron oxidation (Huettel et al., 2014; Huettel & Rusch, 2000).

Spatial variability and environmental drivers of benthic fluxes

Oxygen is important in instigating mineralisation of organic matter and the subsequent recycling of nutrients in the sediment in Macquarie Harbour. This is because bottom water dissolved oxygen was the variable that most consistently appeared to be associated (albeit together with other variables) with predicting most of the flux responses. In addition, different combinations of environmental variables would appear to explain each of the benthic fluxes. The most effective combination of the environmental variables

measured in this study could explain between 45 to 70 % of the variability associated with each flux. This means that there are other drivers not measured in this study that could provide a further understanding of the variability in flux rates.

Sediment median grain size explained a large part of the variation (26.54 %) between sites (not including KR1). Fine-grained sediments generally are less permeable than coarser sediments such as sand, which also means that there is less advective oxygen supply in sediments (Janssen et al., 2005). This can result in shallower oxygen penetration depths compared to coarser sediment and will influence the rates of benthic processes (Cai & Sayles, 1996; Huettel et al., 2014).

KR1 was clearly different from other sites of the harbour based on the large differences in flux rates of ammonium, DIC and DNRA. Benthic mineralisation in KR1 was predominantly anaerobic, as indicated by the relatively high RQ, ammonium efflux and DNRA. There could be a number of reasons why this site appears to have shifted towards anaerobic mineralisation, even with the higher oxygen concentration in the overlying water compared to sites situated in the central harbour. Perhaps the high levels of metals, particularly copper, present in the sediments and water column at this location are influencing the sediment response (Teasdale et al., 1996). KR1 has been highly impacted in the past by mining operations (Koehnken, 1996), with a large amount of dissolved copper still entering Macquarie Harbour via the King River (Teasdale et al., 1996). High concentrations of copper are known to be toxic to many bacteria (Nies, 1999), however, Pavissich et al. (2010) found that excess levels of copper did not inhibit sulfate reduction in sulfate-reducing bacterial communities from sediments with long-term exposure of copper mining residues. The high anaerobic respiration rate measured at KR1 (despite higher bottom water oxygen concentration) indicate that bacteria such as copper resistant sulfate-reducing bacteria may be influencing the microbial community and changing the overall

community structure from one that is responsible for aerobic respiration. The high concentrations of copper in this region of the harbour may have also had a major impact on the abundance of macrofauna (which is on the whole very low to zero as shown in O'Connor et al. (1996) and in this study), as previous studies have shown adverse impacts of copper on many marine invertebrates (Morrissey et al., 1996; Neira et al., 2011; Rygg, 1985) and this in turn could have an associated impact on bioturbation of the sediment. This observation is consistent with that of previous studies in Macquarie Harbour (O'Connor et al., 1996; Talman et al., 1996) where low abundance of benthic organisms were reported due to sediment toxicity caused by copper (Teasdale et al., 2003). Any reduced bioturbation in this region would in turn potentially reduce the amount of sediment exposed to oxygen, and this is consistent with the observation of the low oxygen penetration depth at KR1. The different benthic respiration and nutrient fluxes at KR1 compared to other sites around the harbour demonstrated the sediments response towards a change in environmental condition.

Comparison with other estuaries

Macquarie Harbour's unique physical characteristics limit the extent to which benthic respiration and nutrient flux rates can be compared with other estuaries. According to Edgar and Cresswell (1991) Macquarie Harbour shares similar physical and hydrological characteristics to only one other estuary in Australia, which is the pristine Bathurst Harbour in south western Tasmania. However, there have been no benthic nutrient studies done on Bathurst Harbour for the purpose of comparison. Consequently in order to assess conditions and relative performance of the benthic processes in this system the most appropriate comparison may be with estuaries or fjords in other parts of the world that share similar physical or hydrobiological characteristics, such as long residence time and restricted exchange between the estuary and the open ocean.

The oxygen consumption rates measured in Macquarie Harbour were comparable to the rates previously reported for Fana fjorden, Norway (Wassmann, 1984) and Lochs Creran, Goil, Fyne and Linnhe, Scotland (Overnell et al., 1996) and ammonium flux rates were comparable to those reported for Loch Creran (Table 10). These systems are physically similar to Macquarie Harbour in that they have relatively deep basins with shallow sills and long water residence time. Low bottom water dissolved oxygen concentrations were also a feature in these systems especially in Loch Goil where oxygen levels could fall as low as 0.9 mg/L before bottom water renewal. Similar to Macquarie Harbour, higher oxygen consumption rates as a result of the degradation of riverine organic matter were also measured in the upper regions of the lochs (Overnell et al., 1996). Denitrification and DNRA rates in Macquarie Harbour fell within the range observed in estuaries such as the Gulf of Finland (Jäntti & Hietanen, 2012), where water exchange is restricted and highly eutrophic and where bottom water dissolved oxygen concentration is reduced. Yet, even though the benthic flux rates in these systems are comparable to that of Macquarie Harbour, the physical and biological characteristics and the underlying driver of the flux rates in the harbour may differ from other systems.

Table 10 Comparison of fluxes between Macquarie Harbour and other estuaries.

Location	Depth (m)	Flux (mmol m ⁻² d ⁻¹)		Reference
O ₂ consumption				
Loch Creran, Scotland	15 - 17	8.8 - 23.6		Nickell et al., 2003
Loch Goil, Scotland	44 - 86	15.1 - 23.6		Overnell et al., 1996
Loch Fyne, Scotland	38 -130	10.7 - 20.4		Overnell et al., 1996
Loch Etive, Scotland	26 - 123	8.0 - 52.3		Overnell et al., 1996
Loch Linnhe, Scotland	72 -150	7.2 - 19.0		Overnell et al., 1996
	100	10.3 - 18.4		Overnell et al., 1996
Fanafjorden, Norway	60 - 90	5.3 -10.8		Wassman, 1984
Macquarie Harbour, Australia	7 - 40	2.98 - 17.22		This study
Ammonium Nitrate				
Loch Creran, Scotland	15 - 17	1.29 - 1.40	0.11 - 0.16	Nickell et al., 2003
Loch Linnhe, Scotland	100	0 - 1.5	-0.3 - 0.25	Overnell et al., 1995
Macquarie Harbour, Australia	7 - 40	0.09 - 2.01	-0.41 - 0.004	This study
Denitrification DNRA				
Gullmar Fjord, Baltic Sea	1 - 15	0.04 - 0.36	-	Sundbäck, 2004
Baffin Bay, US	1.8 - 2.2	0.4 - 1.63	0.48 - 1.4	An & Gardner, 2002
Laguna Madre, US	0.8 - 0.9	0.2 - 0.98	0.9 - 3.6	An & Gardner, 2003
Gulf of Finland, Baltic Sea	60 - 83	0.038 - 1.619	0.033 - 1.06	Jäntti & Hietanen, 2012
Macquarie Harbour, Australia	7 - 40	0.055 - 0.215	0.009 - 0.425	This study

Conclusion

The results of this study demonstrated that the Macquarie Harbour sediments are a source of ammonium but a sink for nitrate. Other than coupled nitrification-denitrification, uncoupled denitrification in the sediments seemed to be an important pathway for removal of nitrogen from the overlying water column. The bottom water ammonium and nitrate concentrations would appear to have remained relatively stable over time (see MHDOWG, 2014) and it is hypothesised that the ammonium released from the sediments is most likely being oxidised into nitrate in the water column and absorbed into the sediments for uncoupled denitrification.

Oxygen concentration in the overlying water was one of the most significant factors in determining the individual nutrient and respiration flux rates in Macquarie Harbour. The residence time, and thus renewal and condition of Macquarie Harbour bottom water is influenced by several factors, such as regulated river discharge from the Gordon river dam, weather and tidal conditions (MHDOWG, 2014). Likewise, the supply of organic matter and the concomitant oxygen demand during mineralisation will also influence dissolved oxygen in the bottom waters. The specific role of each of these factors in contributing to the recent decline in dissolved oxygen concentration remains unclear but irrespective of the drivers the results of this study suggest that changing bottom water dissolved oxygen conditions will affect sediment biogeochemistry in the harbour.

Chapter 3: Sediment response to organic enrichment in Macquarie Harbour

Abstract

This study provide the first assessment of the effects of organic enrichment from fish farming on benthic respiration and nutrient cycling in Macquarie Harbour. We compared flux rates at the fish cages, 50 m away from cages and control sites. The flux rates were elevated under the cages as a result of organic enrichment from farming activities. DIC/O₂ ratios especially at the cages indicate that mineralisation was mostly through anaerobic pathways. Sediment characteristics indicated that the fish farming footprint may extend beyond 50 m, however the flux rates at the 50 m sites were not markedly different than the control sites. This suggests that the deposition of organic matter is highly localised. Directly below the cages, denitrification was largely dependent on nitrate sourced from the overlying water column which differs from the control sites where denitrification relied mostly on nitrate sourced from the nitrification of ammonium in the sediments. The response towards fallowing and stocking of the fish cages varied considerably amongst the different leases which showed that other factors may play a significant role in the processing of organic matter.

Introduction

In recent years, the demand for seafood has increased as a result of human population growth and declining wild fish stocks (Diana, 2009; Duarte et al., 2009). In 2012-2013, aquaculture production in Australia accounts for 35% of Australian fisheries and aquaculture production volume. Over the past decade (2004 -2013), salmonid aquaculture in Tasmania has increased its production by 152% to 42 978 tonnes and has become Australia's most valuable fisheries product (Stephan & Hobsbawn, 2014).

The expansion of salmonid aquaculture is contingent on ensuring environmental sustainability. Cage culture can release wastes to the surrounding environments as a result of uneaten feed, fish faeces and excretion (X. Wang et al., 2012). The accumulation of faecal waste and uneaten fish feed can lead to impacts on the benthic environment (Holmer & Kristensen, 1992; Wu, 1995). Several of the reported impacts are high oxygen demand, elevated nutrient regeneration, production of toxic gases and changes in benthic macrofauna communities (Black et al., 2008; Holmer et al., 2005; Wu, 1995).

The highly labile organic matter is rapidly mineralised by bacteria either through aerobic or anaerobic mineralisation. The increase in bacterial activity causes rapid oxygen consumption in the sediment which consequently decreases the oxic zone and increases the anoxic zone where anaerobic processes such as denitrification, manganese reduction, iron reduction and sulfate reduction occur (Glud, 2008; Rysgaard et al., 2001; Sørensen et al., 1979). Anaerobic mineralisation around fish farms tends to dominate the mineralisation process. This is evident as highly reduced sediment conditions under fish farms are well known to appear as black sediment which is from the formation of iron sulfide as a result of iron and sulfate reduction accompanied by a strong smell of hydrogen sulfide and *Beggiatoa* mats (Findlay & Watling, 1997; Hansen et al., 1990; Hargrave et al., 1993; Holmer & Kristensen, 1992; Karakassis et al., 2002).

High oxygen consumption can not only cause a shift in mineralisation pathways but also pose as a risk for hypoxia or anoxia. Such decrease in oxygen concentration could potentially alter nitrogen and phosphorus cycling (Conley et al., 2009; Diaz & Rosenberg, 1995; Sundby et al., 1992). For nitrogen, reduced oxygen concentrations increases the release of ammonium produced via mineralisation since nitrification is inhibited (Cai & Sayles, 1996; Herbert, 1999) and dissimilatory nitrate reduction to ammonium (DNRA) is enhanced (Joye & Anderson, 2008). As a consequence of reduced sediment nitrification,

denitrification, the crucial process that permanently removes nitrogen (Berelson et al., 1998; Seitzinger, 1987), must rely on nitrate sourced from the water column i.e. uncoupled nitrification-denitrification. Furthermore denitrification may be inhibited by the presence of sulfide (An & Gardner, 2002) in highly reduced sediment conditions under cage aquaculture (Bissett et al., 2009; Christensen et al., 2000). For phosphorus, iron bound phosphorus is likely to be released under anaerobic conditions, thereby contributing to the accumulation of phosphate produced through mineralisation of organic matter (Lukkari et al., 2008). Nutrients released from salmon farming together with mineralisation and the release of nutrients from locally deposited organic enrichment contributes to the broader source of nutrients available for primary production.

The magnitude and extent of fish farming effects on the benthic environment also varies depending on the hydrodynamics of the location, production volumes and farm management practices (Crawford et al., 2002; Kalantzi & Karakassis, 2006; Pearson & Black, 2001; Read & Fernandes, 2003). Footprint or zone of impact are often highly localised given sinking velocities of feed and faeces. However, the size of the footprint is influenced by hydrodynamics (Cromey et al., 2002). In an area that is poorly flushed with low water current, greater accumulation of organic matter would be concentrated in a smaller area (Brooks et al., 2002; Sarà et al., 2006). While the disturbances are highly localised, the slow oxygen renewal due to poor flushing along with rapid mineralisation of organic matter pose a higher risk for hypoxia when compared to a well flushed system. Thus it is important that organic matter originating from farming activities are managed such that the biological and chemical oxygen demand does not exceed the rate of oxygen renewal and cause alterations in the major nutrient recycling pathways.

In Tasmania, the industry plans to double its production capacity by 2024 (Anon, 2015). In line with Tasmania's need to increase production to meet demand, fish farming

has to be environmentally sustainable. Impacts from fish farming are of major concern to both farm and environmental managers as it is directly related to fish and ecosystem (i.e. eutrophication) health (Edgar et al., 2005). Management strategies such as fallowing is therefore a common practice to allow sediments the opportunity to recover (Macleod et al., 2006; McGhie et al., 2000). Fallowing is when fish cages are removed or left empty for a period of time. Studies by Macleod et al. (2006) and Macleod et al. (2007) on salmon farms located on the southeast of Tasmania have shown that the period for fallowing to be effective depends on site-specific factors such as farm production, hydrodynamics and impact levels. They found that the benthic environment underneath fish farms with similar stocking levels and feed inputs responded differently during fallowing. Even though the initial impact at a more sheltered area were greater than the exposed ones, the two areas returned to similar levels following the same period of recovery and this is mostly due to local hydrodynamics. The sheltered area was more resilient as it naturally received more organic matter. Meanwhile, at the exposed area, wave and tidal disturbances may have played a role in preventing speedy recovery. In Macquarie Harbour, the effectiveness of fallowing is still not known. As it is a very different system to the salmon farms located on the southeast of Tasmania in terms of residence time, hydrodynamic, and other physical and biological attributes, the same management practices may not apply to the harbour. Therefore, it is important to examine the response of the harbour's benthic environment towards fallowing.

This study provides the first assessment of the effects of organic enrichment from fish farming on benthic respiration and nutrient cycling in Macquarie Harbour, comparing the benthic nutrient flux rates at sites directly adjacent to cages with sites 50 m away and a more distant control sites. To investigate temporal variation and the benthic response to fallowing, the survey was conducted four times. The results are used to verify the extent of

the fish farming footprint in Macquarie Harbour and the effectiveness of management strategies.

Materials and Methods

Study location

Detailed information on Macquarie Harbour can be found in the report by Koehnken (1996). The tidal range in the area is not large (< 0.5 m; (Tong & Williamson, 1998)) and the water column is strongly stratified (Cresswell et al., 1989) due to freshwater inputs from the Gordon and King Rivers and constrictive entrance to the Southern Ocean at Hell's Gate (Tong & Williamson, 1998). The stratified water consists of three distinct layers: a brackish surface layer, a bottom layer with low dissolved oxygen and high salinity, and an intermediate layer which is a mix of the surface and bottom layer (Cresswell et al., 1989). Macquarie Harbour is considered to be poorly flushed. Koehnken (1996) estimated that the marine bottom water has a residence time of about 140 days while the freshwater in the harbour has a residence time of about 70 days. The sediment of the study area is predominantly silt as reported by Lucieer et al. (2009).

This study was conducted in the vicinity of three Atlantic salmon farming zones. Information on the marine farming zones and management control is available in Macquarie Harbour Marine Farming Development Plan (DPIPWE, 2012). Fish farming in Macquarie Harbour commenced in the 1980's and since then a total of 926 ha of maximum leasable area has been approved for fish farming (DPIPWE, 2012). The location for sampling at the fish farm sites in this study are around central harbour in Zone 4A (Liberty Point lease), Zone 7 (Central lease) and Zone 8 (Gordon lease) (Figure 10). The average current velocities

of the entire water column are low around the fish farms ranging from 1.47 to 1.71 cm s⁻¹ during summer and 2.66 to 4.07 cm s⁻¹ during winter (DPIPWE, 2012).

Sampling design

In each of the three farming zones, sediments were collected under 2 fish cages (cage 1 and cage 2), 50 m away from cage 2 and at a control site (0.5 to 1 km away from cage 2). The location of cage 2, 50 m and control sites were chosen to reflect a gradient of effects. It is assumed that maximum impact will occur at the farms and decrease with distance away from the farm. The control and 50 m sites were selected based on having similar depth and sediment type to the farms. Sediments from all three study areas (Gordon, Central and Liberty Point lease) were taken in November '12 and January '13 to investigate the gradient of effects from fish farming activities. Additional samples from Central and Gordon leases were then taken in May '13 and Sep '13 to investigate if there is any temporal differences and sediment recovery associated with fallowing. During the survey period, sediments at the Central lease were stocked in November and January and left to fallow in May and September. At the Gordon lease, cage 1 was stocked the whole time while cage 2 was fallowed in November, January and May then restocked in September. Depths of the sampling sites ranged between 18 m and 37 m.

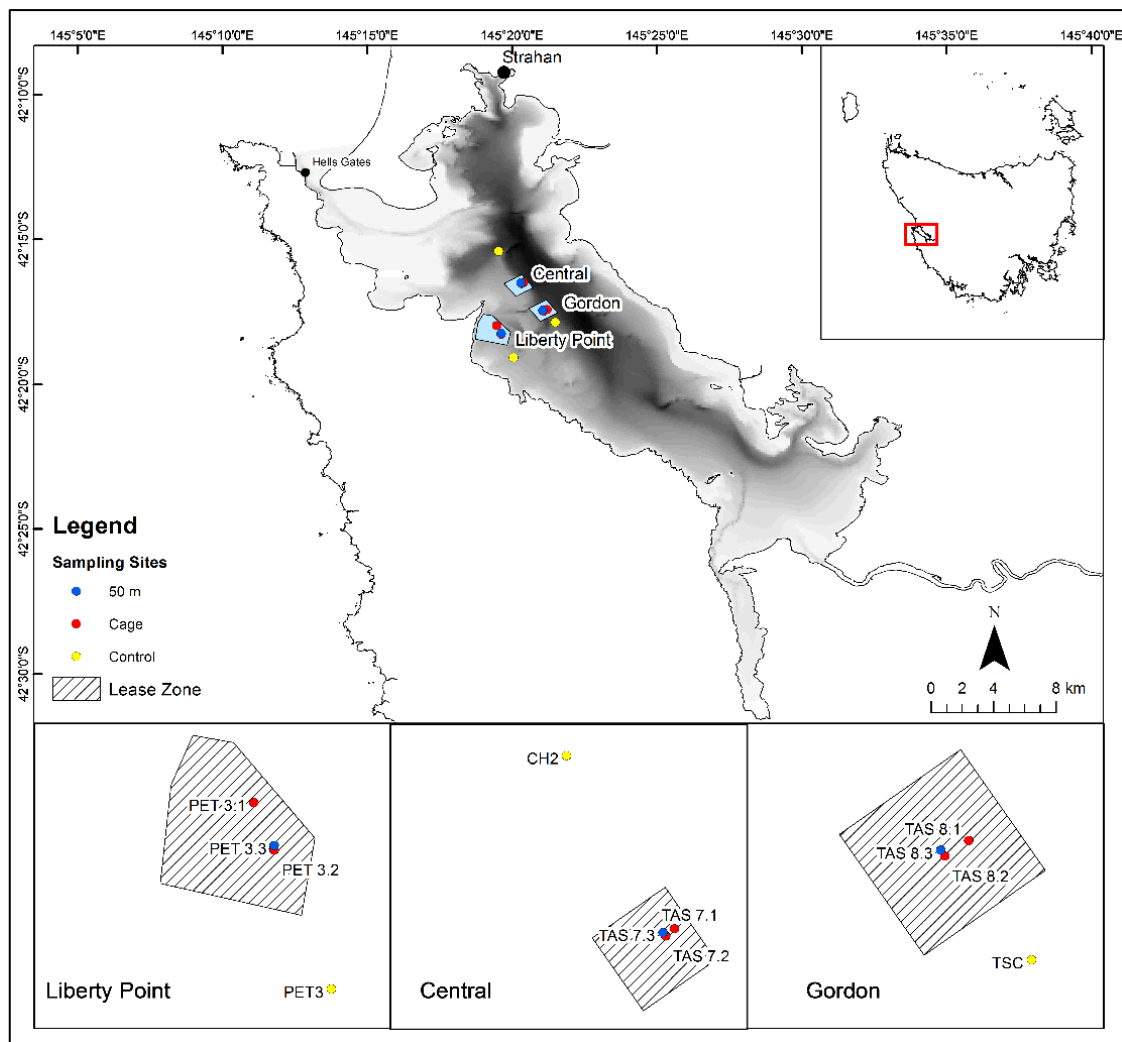


Figure 10 Map of sampling locations. Map only shows fish farming leases where sampling sites were located.

Sample collection

A detailed description of the sampling methods are given in Chapter 2. In summary, four replicate box cores with a Perspex liner were collected at each site. For measuring nutrient fluxes the box core was sub sampled by gently placing 1 large core (300 mm x 150 mm diameter) into the sediments and the bottom immediately capped to prevent any disturbance. For denitrification and DNRA, a sub sample was taken from each of the box cores with a smaller sized core (300 mm x 44 mm diameter). The cores were filled with bottom water collected from approximately 1 m above the sediment using a pump at each sampling area before returning to the laboratory. The top 3 cm of the sediments from each grab were also sampled with a cut off syringe and stored in sample bags (Nasco WHIRL-PAK) for porosity and grain size analysis. Temperature, salinity and dissolved oxygen concentration readings of bottom water at each sampling site were recorded for further comparison of environmental conditions.

Sediment-water nutrient fluxes

As described in detailed in Chapter 2, sediment cores were first immersed in a plexiglass tank filled with bottom water collected at each site and kept at *in situ* temperatures. The next day, the cores were flushed with fresh site water and then sealed to begin the incubation. Incubations were conducted in the dark to reflect *in situ* dark conditions due to the dark tannin rich surface layers. Dissolved oxygen measurements and water samples for dissolved nutrient (ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and filterable reactive phosphorus (measured as PO_4^{3-}) and dissolved inorganic carbon (DIC) were taken 3 or 4 times in a time series. Plastic syringes were used to collect water samples which were then filtered through a 0.45 μm filter (Minisart-plus; Sartorius AG, Goettigen, Germany) then stored in 50 ml polyethylene vials. All nutrient samples were immediately

frozen until analysis. Samples for DIC were kept in 12 ml glass vials (Exetainer; Labco, High Wycombe, UK) and preserved with 20 μ l of Mercuric (II) chloride and refrigerated until analysis. The length of incubation was determined by the total lowering rate of oxygen concentrations by 10 – 20 % of air saturation (Dalsgaard et al., 2000). The flux of each analyte was calculated using linear regression of 3 to 4 data points.

All nutrient samples were analysed by the Water Studies Centre (WSC, Monash University) using flow injection analysis (FIA) (Lachat Quikchem 8000 Flow injection Analyser, spectrophotometric detector). DIC was analysed based on Coulometric method using a LI-7000 CO₂/H₂O gas analyzer (LI-COR Biosciences, Lincoln, NE, USA).

Denitrification and dissimilatory nitrate reduction to ammonium (DNRA)

Rates of denitrification were measured using isotope pairing technique (L. P. Nielsen, 1992) as described by Dalsgaard et al. (2000). This method was used to allow calculation of both coupled and uncoupled nitrification-denitrification. Prior to incubation, samples were taken before and after the addition of 200 μ L of labelled $^{15}\text{NO}_3^-$ to calculate the accumulation of $^{15}\text{N-NO}_3^-$. The cores were then capped for incubation by allowing the $^{15}\text{NO}_3^-$ to diffuse towards the denitrification zone and reach equilibrium. The measurements of N₂ were carried out as a time series and each time one core was sacrificed by adding 1 ml of 50% ZnCl₂ into the cores then homogenised by stirring gently with a glass rod. These cores were left to settle for before samples were taken then preserved in glass vials containing 250 μ l of ZnCl₂ 50% w:v until analysis.

To determine DNRA rates, samples for $^{15}\text{N-NH}_4^+$ were collected from the last remaining core at the end of the denitrification incubations. The rates were calculated based on the linear increase in the amount of $^{15}\text{N-NH}_4^+$ produced during the incubation over time as described in Roberts et al. (2012).

Sediment properties

At the end of the nutrient flux incubation, the upper 3 cm of sediments were collected from two cores using a 60 ml cut-off syringe for sediment porosity, isotopic carbon ($\delta^{13}\text{C}$), isotopic nitrogen ($\delta^{15}\text{N}$), total organic carbon (TOC) content, total nitrogen (TN) content and particle size distribution (PSD) measurements. Sediment porosity was determined from weight loss after oven-drying samples at 105°C to a constant weight. The median grain size (D_{50}) and percentage of particles from the total within the size range for silt/clay ($<40\ \mu\text{m}$) were determined from particle size distribution analysis using laser diffraction method in Saturn Digisizer 5200 (Micromeritics Instrument Corp., USA). Samples for TOC and TN contents and isotopic composition were finely ground and treated to remove inorganic carbon which were then sent to analysis on an ANCA GSL2 elemental analyser interfaced to a Hydra 20-22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd., UK) at the Water Studies Centre.

Statistical analyses

Permutational multivariate analysis of variance (PERMANOVA) was first conducted to verify that the control sites used in this experiment were not significantly different from areas further away from the impact of fish farming activities. Since PERMANOVA performs well for balanced designs (Anderson & Walsh, 2013), three sites located within central harbour (WH2, CHE, CH1; see Chapter 2) and the three sites used as controls for this experiment (CH2, TSC and PET 3) were tested for differences using the type of sites as a factor. As the factor only contained 2 groups, a pair-wise test was used for comparison. This analysis was based on Euclidean distances of untransformed and normalised benthic flux data. Type III sum of squares and unrestricted permutation of raw data using 999 permutations were applied (Anderson et al 2008).

Univariate analysis

A randomised complete block design (RCBD) analysis of variance (ANOVA) was used to determine significant differences in rates of benthic processes between the cage, 50 m and control sites (response variable vs. distance from source of impact) and between each sampling period (response variable vs. sampling times). RCBD was used to reduce spatial effects among the farming areas on the factors distance and time. Because cage 2, the 50 m and control sites were effectively aligned in a transect, for the purpose of having a balanced design, cage 1 from all 3 fish farming area were left out of the ANOVA analysis. Instead, data obtained from cage 1 and cage 2 from all lease area were compared in a descriptive manner.

As initial analysis demonstrated a significant distance by time interaction, and as such, RCBD was used to analyse each individual factor separately. The fixed factors were distance and sampling time with farms as a random blocking factor. To determine the effect of distance from the source of impact on the response variables, analysis was performed on the measurements taken in November and January at the 3 farm leases, while the temporal effect was analysed with measurements taken in November, January, May and September at sites located at the Central and Gordon leases. Assumptions were verified using Levene's test for homogeneity of variance and Shapiro-Wilks test and graphical method by plots of residuals against predicted values for normality of residuals (Quinn & Keough, 2002). The data was transformed when the assumptions were violated and the analysis repeated. Statistical analyses were performed using SAS[®] software (University Edition, SAS Institute Inc.).

Multivariate analysis

Canonical analysis of principal coordinates (CAP) was also performed to look at the relationships of the rates of the benthic processes (response) with environmental variables

(predictor). For this analysis, the average value for each environmental variable and benthic flux rate were used. Data used in this analysis includes all available information for cage 1, cage 2, 50 m and control sites. Sites containing missing information were taken out of the analysis. Prior to the analysis, homogeneity of each variable was assessed using draftsman plot and all data except for coupled nitrification-denitrification (Dn) and total denitrification were transformed. After transformation, the data was also “normalised” as a standardisation routine in PRIMER v6 (Clarke & Gorley, 2006). Both PERMANOVA and CAP analyses were performed using the software package PRIMER v6 and PERMANOVA+ (PRIMER-E, United Kingdom). To look at the relationship between selected environmental variables and fluxes, scatter plots and Pearson correlation were used to present the correlation.

Results

The control sites (CH2, TSC and PET3) in this experiment were not significantly different from sites further away from the fish farming areas (P -perm = 0.52).

Sediment characteristics and bottom water dissolved oxygen concentration

All of the sediments were classified as silt, with grain sizes less than $<40\ \mu\text{m}$ (Table 11). Median grain size at the lease sites did not differ markedly but the percentages of silt/clay were found to be lower at the fish cages (

Table 11). Porosity of the sediments were higher in November compared to other months. However, there was no pattern with distance from the fish cages in any of the areas.

Bottom water dissolved oxygen (BDO) concentrations were generally higher in November and January compared to May and September. In May and September, BDO concentrations at Central and Gordon leases dropped to levels below < 1.0 mg/L. BDO concentration varied from around 0.61 to 1.2 mg/L. No obvious trend was observed with distance from the cage sites at all farm leases.

The carbon ($\delta^{13}\text{C}$) signatures generally decreased with distance from the fish cages (Figure 2). At the fish cages, $\delta^{13}\text{C}$ values were higher (-23.76 to -26.17 ‰) compared to both sites 50 m away (-25.55 to -27.31 ‰) and control sites (-26.86 to -27.89). The same trend was observed for nitrogen ($\delta^{15}\text{N}$) signatures. At the 50 m and control sites, most of the $\delta^{15}\text{N}$ values were <5 ‰, whereas the cages had values ranging from 5.16 to 7.45 ‰. The total organic carbon (TOC) and total nitrogen (TN) contents from surficial sediments showed a similar pattern to that of the isotope signature values, and were generally higher at the cage sites, this trend was particularly obvious at the Central lease (Figure 12). At the 50 m site, TOC and TN contents were markedly lower than at the cage sites, but still higher than the levels at the control sites (Figure 12). At the Gordon and Liberty Point leases, TOC contents did not vary as much as it did at the Central lease, with values ranging from 0.07 to 0.11 % and 0.09 to 0.11 % respectively. TN content was slightly elevated at the cage sites but decreased with distance.

C/N molar ratios at all control sites were higher than at 50 m and cage sites, with values ranging between 19.26 and 23.87. At the 50 m sites, the ratios varied between 9.53 and 14.80, except at Liberty Point where the value was higher (19.75) compared to the 50 m

sites at Central and Gordon leases. At the cage sites, the ratios range between 6.14 and 13.51.

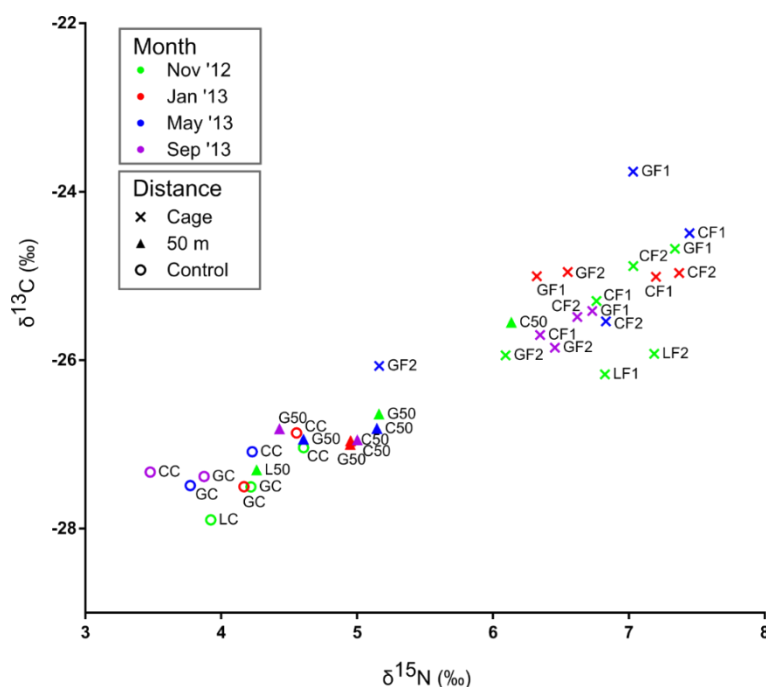


Figure 11 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures from surficial sediments taken at four sampling times. CF1: Central lease cage 1; CF2: Central lease cage 2; C50: Central lease 50 m; CC: Central lease control; GF1: Gordon lease cage 1; GF2: Gordon lease cage 2; G50: Gordon lease 50 m; GC: Gordon lease control; LF1: Liberty Point lease cage 1; LF2: Liberty Point lease cage 2; L50: Liberty Point lease 50 m; LC: Liberty Point lease control.

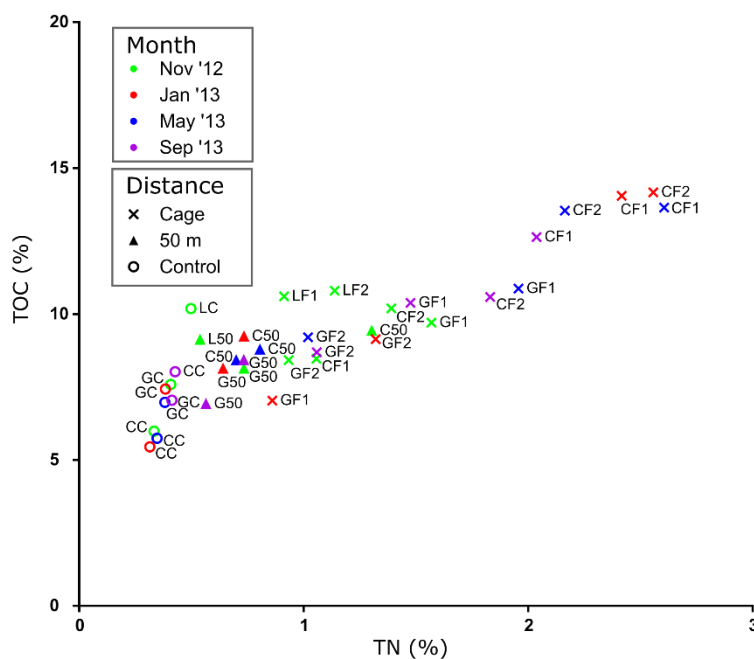


Figure 12 Percentage of total organic carbon (TOC) and total nitrogen (TN) content in sediments taken from cage, 50 m and control sites in Macquarie Harbour. CF1: Central lease cage 1; CF2: Central lease cage 2; C50: Central lease 50 m; CC: Central lease control; GF1: Gordon lease cage 1; GF2: Gordon lease cage 2; G50: Gordon lease 50 m; GC: Gordon lease control; LF1: Liberty Point

lease cage 1; LF2: Liberty Point lease cage 2; L50: Liberty Point lease 50 m; LC: Liberty Point lease control.

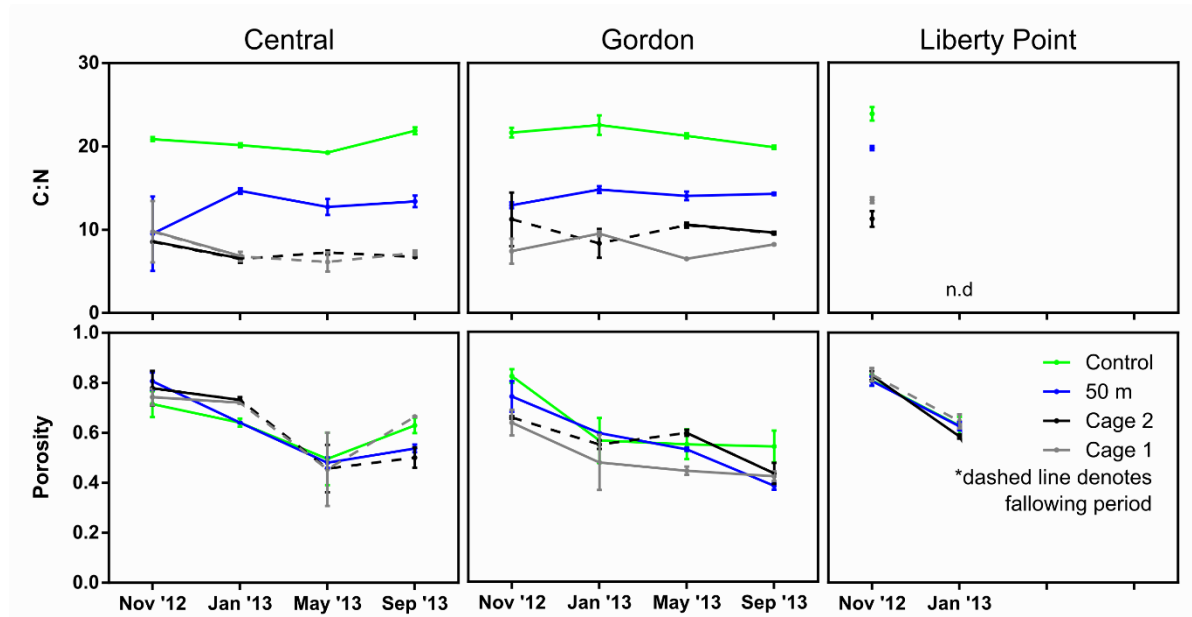


Figure 13 Differences in C/N molar ratio and porosity among sites. Error bar on each point represent standard deviation with n=2.

Table 11 Sediment characteristics (bottom water dissolved oxygen concentration (BDO), grain size and depth) of sediments taken from 3 different farm leases in Macquarie Harbour.

Distance	Month	BDO (mg/L)	Grain Size (μm)	Silt/Clay (%)	Depth (m)	BDO (mg/L)	Grain Size (μm)	Silt/Clay (%)	Depth (m)
Central					Gordon				
Cage	Nov	1.70	5.94	84.40	36	1.60	8.89	92.30	36
	Jan	2.60			36	2.30			36
	May	0.40			35	0.98			31
	Sep	0.46			32	0.55			31
Cage	Nov	1.10	15.12	81.30	26	1.30	10.49	93.60	36
	Jan	1.20			26	2.40			36
	May	1.02			25	0.93			33
	Sep	0.59			25	0.56			33
50m	Nov	1.30	10.79	90.50	26	1.30	11.01	93.90	35
	Jan	1.90			26	2.10			35
	May	1.09			25	0.83			35
	Sep	0.90			25	0.21			35
Control	Nov	1.90	6.74	98.80	35	2.20	9.47	97.20	32
	Jan	3.30			35	2.70			32
	May	1.61			37	0.60			33
	Sep	0.70			37	0.72			33
Liberty Point									
Cage	Nov	0.90	12.56	91.50	18				
	Jan	0.63			18				
Cage	Nov	0.80	12.19	91.30	20				
	Jan	0.61			20				
50m	Nov	0.80	11.03	94.50	18				
	Jan	0.62			18				
Control	Nov	1.20	11.54	97.20	18				
	Jan	0.64			18				

Benthic flux rates

There were no significant differences between the different sampling times at either the cage, 50 m or control sites for sediment oxygen (O_2), nitrite (NO_2^-), nitrate (NO_3^-), uncoupled nitrification-denitrification (Dw), coupled nitrification-denitrification (Dn), total denitrification and dissimilatory nitrate to ammonium (DNRA) fluxes (Table 12 and Table 13). In November, O_2 flux did not differ significantly with distance from the fish cages (-6.99 to -28.63 $mmol\ m^{-2}\ d^{-1}$). However in January, O_2 consumption at the fish cages (-18.37 to -70.80 $mmol\ m^{-2}\ d^{-1}$) were significantly higher than that at either the 50 m (-3.91 to -24.42 $mmol\ m^{-2}\ d^{-1}$) and control sites (-3.98 to -11.03 $mmol\ m^{-2}\ d^{-1}$), notably at the Central lease (

Table 14). Similar patterns of response were observed at the Central and Gordon leases for dissolved inorganic carbon (DIC) and phosphate (PO_4^{3-}) in January. DIC fluxes were significantly higher at the cage sites than at other stations, with flux rates ranging between 171.64 and 372.67 $\text{mmol m}^{-2} \text{d}^{-1}$. The 50 m (9.80 to 26.17 $\text{mmol m}^{-2} \text{d}^{-1}$) and control sites fluxes (5.37 to 7.75 $\text{mmol m}^{-2} \text{d}^{-1}$) were not significantly different. Fluxes of DIC and PO_4^{3-} were consistently higher at the cage sites than elsewhere in May and September, even though there were not significantly different between sampling times. DIC fluxes in November did not differ significantly with distance from the cages while PO_4^{3-} efflux from the sediment to the water column was significantly higher at the cage sites. In fact PO_4^{3-} fluxes at the 50 m and control sites were mostly directed from the water column into the sediment.

The release of NH_4^+ from the sediment was significantly different between the cage, 50 m and control sites, both in November and January whereas NO_2^- fluxes did not differ between the sites over this sampling period. At the control sites, NO_3^- uptake rates were significantly lower than at either the cage or 50 m sites, which did not differ significantly from each other. In November, denitrification and DNRA rates were only measured at the Gordon and Liberty Point leases and were similar at that time. Denitrification and DNRA rates measured in January were also not significantly different between sites, but denitrification rates appeared to decrease with distance from cages. Denitrification, based on D_w was higher at the cage and 50 m sites compared to the controls, where the rates suggested D_n . There was also a site specific difference; with rates of DNRA being higher at the cage site at Central lease, while the DNRA rates at the Gordon and Liberty Point were similar at 50 m and cage sites.

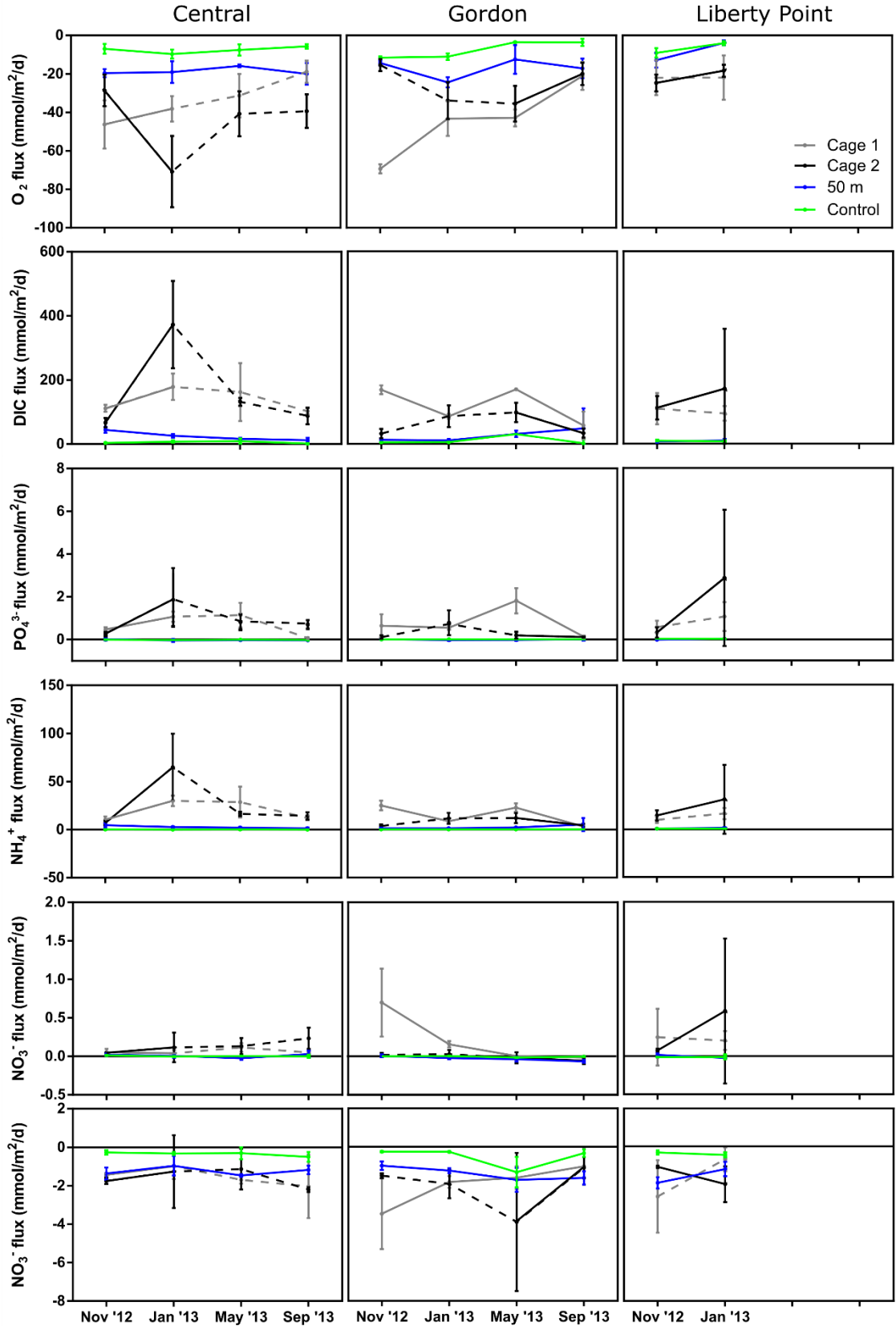


Figure 14 Benthic flux rates of oxygen (O₂), dissolved inorganic carbon (DIC), phosphate (PO₄³⁻), ammonium (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻). Each point represent average values and error bar for standard deviation of four replicates. Positive values indicates release from sediment and negative values indicates uptake by sediment.

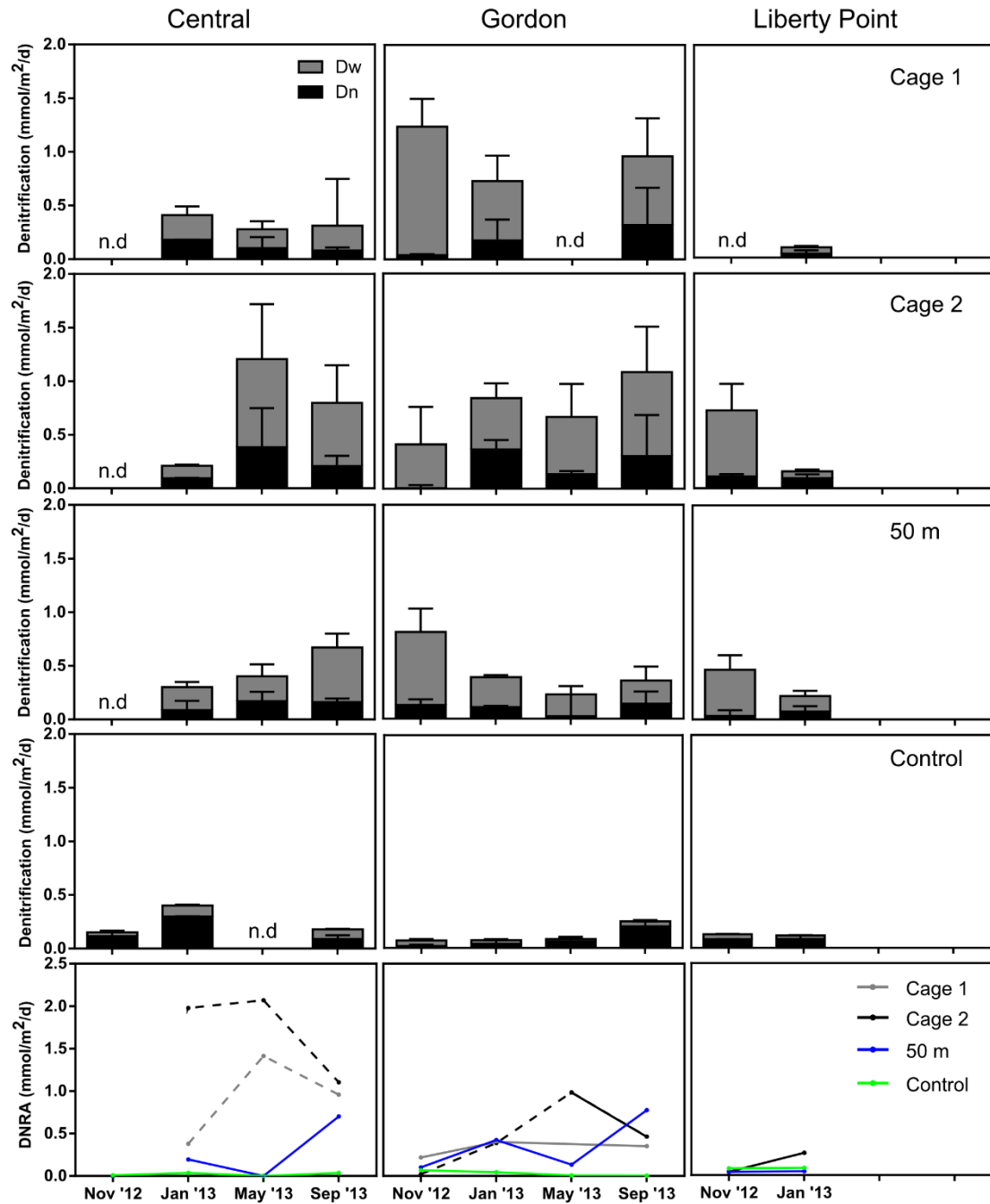


Figure 15 Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) rates measured in Macquarie Harbour. Dw represents the process of uncoupled nitrification-denitrification and Dn represents coupled nitrification-denitrification. Error bar on each bar and point indicates standard deviation.

Table 12 Results of one-factor ANOVA of RCBD on the sampling times at cage and 50 m sites from Central and Gordon leases. Significance level used were p -value < 0.05 .

Variable	Cage		50m	
	Transformation	P -value	Transformation	p -value
O ₂	log(x)	0.1543	x	0.3219

DIC	$\log(x+0.1)$	0.0661	$x^{1/3}$	0.4257
PO_4^{3-}	$\log(x+1)$	0.055*	$\log(x)$	0.2344*
NH_4^+	x	0.093	x	0.8724
NO_2^-	$x^{1/3}$	0.9137	x	0.4892
NO_3^-	x	0.7052*	x	0.3641
Dw	x	0.2388	x	0.8301
Dn	x	0.9858	x	0.4416
Denitrification	x	0.6132	x	0.6732
DNRA	x	0.2907	x	0.9139

*Indicates that normality of residuals analysed using Shapiro-Wilks test was not met

Table 13 continued Results of one-factor ANOVA of RCBD on the sampling times at control sites from Central and Gordon leases. Significance level used were p-value <0.05.

Variable	Control	
	Transformation	p-value
O ₂	$\log(x+0.1)$	0.2698
DIC	x	0.4555
PO_4^{3-}	$\log(x+0.1)$	0.7164*
NH_4^+	$\log(x+0.05)$	0.1457
NO_2^-	$\log(x+0.03)$	0.5485*
NO_3^-	x	0.5502*
Dw	x	0.6879
Dn	x	0.7566
Denitrification	x	0.6911
DNRA	x	0.558

*Indicates that normality of residuals analysed using Shapiro-Wilks test was not met.

Table 14 Results of one-factor ANOVA of RCBD on distance using measurements taken in November and January from Central, Gordon and Liberty Point leases. Significance p -values (<0.05) are shown in bold.

Month	Variable	Transformation	p -value	Tukey
Nov	O ₂	log(-x)	0.0617	
	DIC	log(x)	0.0568	
	PO ₄ ³⁻	$x+0.5^{1/3}$	0.0100*	Cage <u>50m</u> Control
	NH ₄ ⁺	log(x+0.1)	0.0253	Cage, 50m, Control
	NO ₂ ⁻	None	0.0772	
	NO ₃ ⁻	$x^{1/3}$	0.0003	<u>Cage 50m</u> , Control
Jan	O ₂	log(-x)	0.0170	<u>Cage 50m</u> Control
	DIC	log(x)	0.0007	Cage, <u>50m</u> Control
	PO ₄ ³⁻	$x^{1/3}$	0.0004	Cage, <u>50m</u> Control
	NH ₄ ⁺	log(x+1.5)	0.0118*	Cage, 50m, Control
	NO ₂ ⁻	log(x+0.1)	0.1333*	
	NO ₃ ⁻	$x+0.5^{1/3}$	0.0009*	<u>Cage 50m</u> , Control
	Dw	x	0.3071	
	Dn	x	0.8097	
	Denitrification	x	0.3071	
	DNRA	x	0.2562	

*Indicates that normality of residuals analysed using Shapiro-Wilks test was not met

DIC flux was strongly correlated with both NH₄⁺ (Pearson $R = 0.9673$, $P < 0.05$) and PO₄³⁻ ($R = 0.8187$, $P < 0.05$) (Figure 16). The relationship between DIC and NH₄⁺ was not so clear at the 50 m ($R = 0.6268$, $P > 0.05$) and control sites ($R = 0.4500$, $P > 0.05$), compared to the fluxes at the cage sites ($R = 0.9519$, $P < 0.05$). The correlation between DIC and PO₄³⁻ was also stronger at the cage sites ($R = 0.7388$, $P < 0.05$), but the fluxes at the 50 m and control sites were not significantly correlated. DIC was positively correlated with DNRA ($R = 0.6687$, $P < 0.05$) and negatively correlated with O₂ flux ($R = -0.7403$, $P < 0.05$).

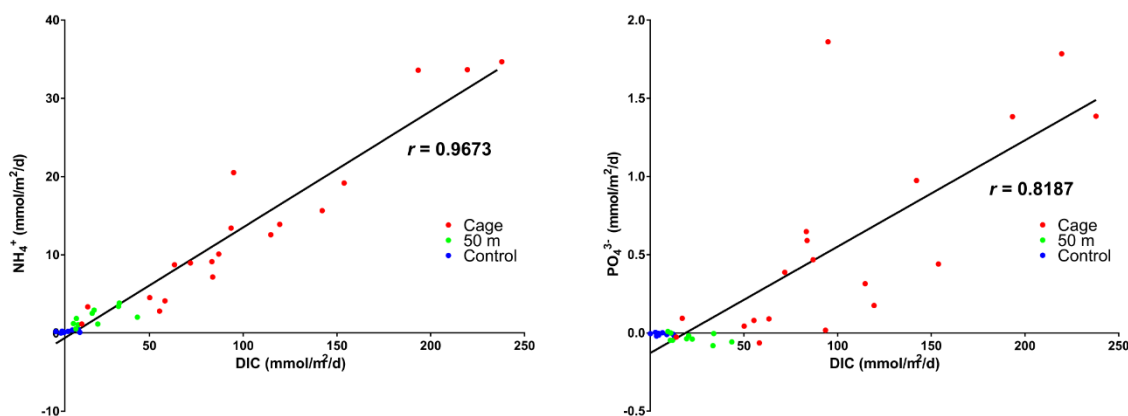


Figure 16 Relationship between DIC relative to NH_4^+ and PO_4^{3-} . The Pearson correlation coefficient (r) is given in the plot.

Comparison between cages

The fish farming leases had different stocking and fallowing periods during the study period. The O_2 consumption rate at both cage sites within the Central lease decreased during fallowing (Figure 14). In January, at cage 1, O_2 consumption rate decreased when compared to the consumption rate in November even when the cage was stocked. In contrast, O_2 consumption rate increased two-fold at cage 2 in January compared to November. Observation of the other fluxes indicate that when the cages were stocked DIC, PO_4^{3-} and NH_4^+ flux rates were higher compared to fallowing period while the NO_3^- flux rate decreased.

At the Gordon lease, O_2 , DIC, NH_4^+ and NO_3^- flux rates at cage 1 all increased during fallowing period and decreased after restocking (Figure 14). There was no obvious pattern in these nutrients at the stocked cage 2 with the exception of O_2 and NO_3^- flux rates which decreased with time. Benthic flux rates at Liberty Point's cage 2 generally increased after stocking, except for O_2 which decreased from November to January. Meanwhile, fluxes at cage 1 declined during fallowing in January except for PO_4^{3-} and NH_4^+ .

Exploring relationships between fluxes and environmental variables

The CAP result shows that there were very strong and significant correlations between the fluxes data cloud (based on Euclidean distances) and the measured environmental variables ($P = 0.0001$). The first canonical correlation was high ($\delta_1 = 0.9718$), while the second canonical correlation was $\delta_2 = 0.7321$ (Figure 17). The first 2 PCO axes explain 94.45% of the total variability in the resemblance matrix. The CAP analysis clearly separated the cages, 50 m and control sites. C/N molar ratio had the highest canonical eigenvector value, indicating that this variable had the largest effect on the multivariate distribution (CAP1). Percentage of silt/clay and porosity were more strongly correlated with the sample site distribution (correlated with CAP2). Observation of the multivariate pattern showed no clear temporal differences.

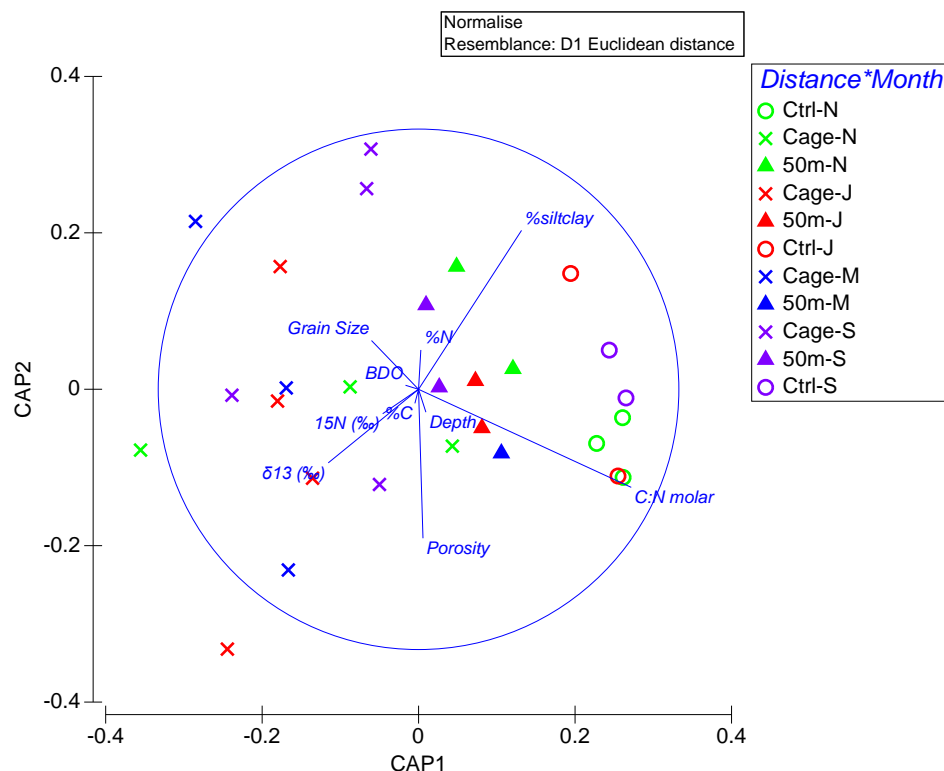


Figure 17 CAP analysis of variation in flux measurements of sediment samples collected from cage, 50 m and control sites at Central, Gordon and Liberty Point leases. Vectors represents correlations of environmental variables to the variation in the benthic fluxes.

Scatter plots (Figure 18) of C/N molar ratio, TOC and TN content relative to DIC and NH_4^+ reveal strong inverse correlations between these variables, and distinct separation of sites with distance away from the cage. Smaller C/N molar ratios were correlated to higher DIC and NH_4^+ fluxes, while higher OC and TN levels in the sediment were correlated with higher DIC and NH_4^+ loads.

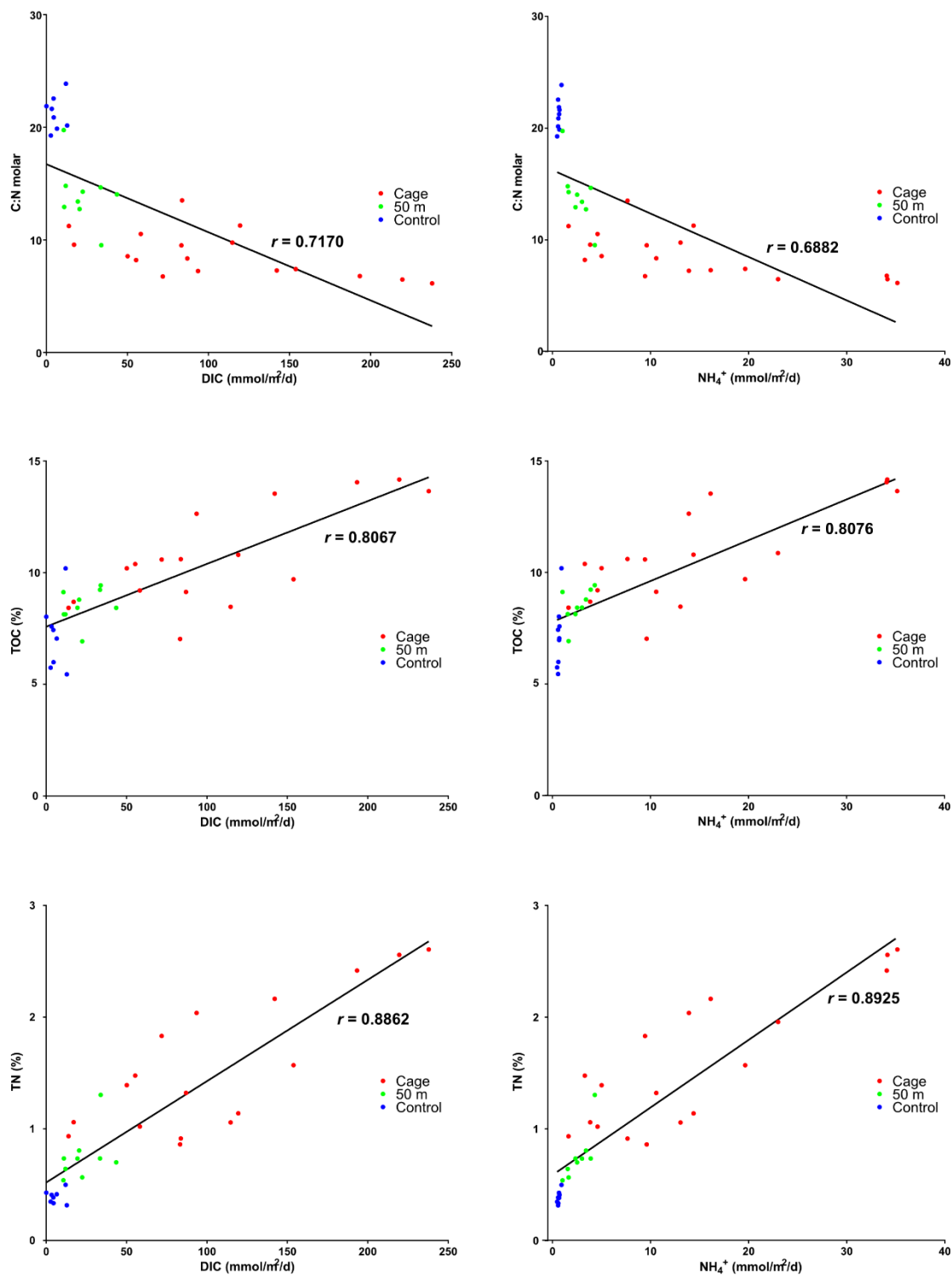


Figure 18 Pearson correlation analysis between DIC and NH₄⁺ with several selected environmental variables which are C:N molar, TOC and TN content. Pearson correlation coefficient are (r) was also given in the plot.

Discussion

Variability in sediment characteristics

There was a clear effect of farming at cage sites in Macquarie Harbour based on all of the measures of organic enrichment assessed in this study; with the result observed being broadly consistent with that previously reported from other salmon farming regions in the southeast of Tasmania (Macleod et al., 2004). However, there were subtle differences in many of the individual measures of organic enrichment that provide an understanding on the small scale variability within the selected study sites (leases).

Previous studies have reported marked differences in the spatial extent of organic enrichment associated with fish farming; Karakassis et al. (1998) and McGhie et al. (2000) found that the farthest effects were only 20 to 25 m away from the fish cages, whereas Yokoyama et al. (2006) and Sarà et al. (2006) found effects up to 300 m and 1000 m from the cages respectively. There may be a number of reasons for these differences, including but not limited to, production intensity, water depth and current velocities. Areas with low current velocities tend to limit the range of organic matter deposition, for example the depositional footprint of waste material in the Gulf of Castellammare (a location with low flow; mean velocity of current $\sim 12 \text{ cm s}^{-1}$) was less than 100 m away from fish cages (Sarà et al., 2006). In Macquarie Harbour, the flow rate around the farm sites was between 1.47 to 4.07 cm s^{-1} all year round, and there was a deposition footprint with measures of organic enrichment (TOC, $\delta^{13}\text{C}$, TN, $\delta^{15}\text{N}$, C/N molar ratio) at 50 m to some extent higher than the levels at control sites but markedly lower than at the cage sites. Whilst the full extent of dispersal of material from farm origin in Macquarie Harbour is still not clear, given the low current velocities it might be expected that the impacts would be relatively constrained, i.e. to within 100 m.

In the current study the sediments close to the fish cages were clearly enriched with both carbon (TOC, $\delta^{13}\text{C}$) and nitrogen (TN, $\delta^{15}\text{N}$) and had low C/N molar ratios, especially at Central lease. This study showed a marked decrease in TOC content with distance away from the farm, resulting in a clear “footprint”. In many studies sedimentation rate has been proposed as an important measure of the likely impact associated with fish farming as this can provide an understanding of the overall load to the sediments but it is important to note that sedimentation of carbon is generally spatially constrained compared to nitrogen (Sarà et al., 2006; Schendel et al., 2004) and may not be a good indicator of the actual dispersal of organic matter resulting from fish farming activities.

The isotopic signatures clearly suggested that the organic enrichment at the fish cages were derived from fish farming waste. The levels of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the fish cages were clearly marine based and were consistent with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ levels previously reported for fish feed used in Macquarie Harbour (i.e. -19.7 ± 0.2 ‰ and 7.4 ± 0.2 ‰ respectively (Abrantes et al., 2010)). This contrasted strongly with the sediments at the control sites which had more terrestrial signature. These findings are in agreement with similar studies from Tasmania (McGhie et al., 2000), Europe (Holmer et al., 2007; Sarà et al., 2006) and Asia (Yokoyama et al., 2006) which also showed marked increases in enrichment under or close to cages and defined the materials as fish farm in origin. Consequently isotopic signatures may be a useful measure if determination of source or causality is the priority.

The results of the CAP analysis suggest that the C/N molar ratios could be a useful indicator of the organic enrichment footprint from fish farming in Macquarie Harbour. The C/N molar ratios were strongly correlated with DIC and ammonium flux rates. At the control sites the C/N molar ratios were generally above 19, whilst at the cage sites at the Central and Gordon leases the C/N molar ratios were much lower (between 6.14 to 10.52) and although slightly increased at the 50 m sites the ratio was still only between 12.74 and

14.79. Other studies have also suggested that C/N molar ratio might be indicative of impact, and found similar C/N molar ratios under the net cages (7.5 to 9.0) in Kolding Fjord, Denmark (Holmer & Kristensen, 1992) and in Loch Creran, Scotland (8.08) (Nickell et al., 2003). However, it is important to note that there were temporal differences in the C/N molar ratio response, with the distinction between the cage and 50 m sites only evident in the months of January, May and September, but not in November.

Benthic nutrient processing

When looking at the actual ecological effects of fish farming in Macquarie Harbour it was clear that the higher organic content at the fish cages caused significant increases in oxygen flux rate and production of DIC compared to 50 m and control sites. Oxygen consumption rates were six times higher at the cage sites compared to the controls. This is due to the labile fish farming waste being rapidly mineralised (Valdemarsen et al., 2009). Enhancement of benthic flux rates beneath fish farms has been well documented in temperate coastal regions (Bissett et al., 2006; Christensen et al., 2003; Christensen et al., 2000; Hall et al., 1990; Hall et al., 1992; Hargrave et al., 1993; Holmer & Kristensen, 1992; Nickell et al., 2003; Papageorgiou et al., 2010). This enhancement reflects the activity of the microbial community in assimilating the additional organic material and is a good thing provided the assimilative capacity is not overwhelmed.

Oxygen consumption is a useful indicator of environmental condition as it is an important ecological process, the oxygen regime will define the biological function of the sediments. The greatest increase in oxygen consumption rate in Macquarie Harbour seemed to be localised under the fish cages - oxygen flux rates were up to 6 times higher at the fish cages - rates at the 50 m sites appeared to be slightly elevated, but were not significantly higher than at the control sites. However, oxygen flux rate alone may not fully capture

carbon mineralisation as anaerobic mineralisation can occur when oxygen is limited. DIC flux rates gave a better insight into total carbon oxidation in Macquarie Harbour as it is a measurement of both aerobic and anaerobic mineralisation (Hopkinson & Smith, 2004; Valdemarsen et al., 2009). DIC flux rate relative to oxygen consumption ratio has been used to identify the dominant mineralisation pathway, with a DIC/O₂ ratio of <1 indicating predominantly aerobic mineralisation and a DIC/O₂ ratio of >1 suggesting anaerobic mineralisation (Holmer et al., 2003; Cathalot et al 2012). The DIC/O₂ ratio in sediments beneath fish cages in Macquarie Harbour was generally >2 which indicates that anaerobic mineralisation was dominant. The highest DIC production compared to oxygen consumption was at Cage 2 on Liberty Point lease in January (ratio = 9.3). Anaerobic mineralisation was not limited to the sediments beneath the fish cages and actually appeared to be quite common throughout the harbour (Chapter 2). Sediment from the 50 m station as well as some of the control sites also had DIC/O₂ ratios of >1. Other studies have also reported the presence of sulfate reducing bacteria in Macquarie Harbour (Carpenter et al. (1991). The fact that low oxygen conditions were identified throughout the harbour has conflicting implications for interactions with fish farming: whilst it means that anaerobic conditions are not foreign to the system, there is the potential to place additional stress on what might be considered an already stressed system.

Denitrification is the principal means of removing nitrogen for marine and coastal systems; nitrogen is a key concern with fish farming as it is a major input and has the potential to drive eutrophication. Consequently, measures of denitrification and how that might vary within a system are important as they provide an understanding of the system's capacity to cope with fish farming inputs and therefore will provide important information for management. Although denitrification rates appeared higher at the fish cages (mean = 0.64 mmol m⁻² d⁻¹) and 50 m (mean = 0.42 mmol m⁻² d⁻¹) compared to control sites (mean =

0.16 mmol m⁻² d⁻¹), unfortunately due to the replication level these differences were not significant, and rates did not differ over time. Denitrification rates at Macquarie Harbour were similar to those observed from Horsens Fjord, Denmark (0.02 to 4.00 mmol m⁻² d⁻¹) (Christensen et al., 2000), and Tasman Bay (0.72 to 1.11 mmol m⁻² d⁻¹) and Beatrix Bay, New Zealand (0.19 to 0.26 mmol m⁻² d⁻¹) (Christensen et al., 2003): all similar areas where fish farming is undertaken. Whilst previous studies have shown that organic enrichment seemed to stimulate denitrification activity (Caffrey et al., 1993; Christensen et al., 2000), increasing organic matter inputs, particularly at the fish cages, could reduce denitrification rate, as nitrification is restricted by the lack of oxygen (Henriksen & Kemp, 1988). Eventually, nitrate sourced through nitrification, and from the overlying water column would decrease over time as it is taken up via the denitrification process or reduced as a result of competition with DNRA process, thus decreasing the efficiency of nitrogen removal (Caffrey et al., 1993) and (Christensen et al., 2000). This could be the case at Cage 2 on the Central lease where higher organic content and rapid mineralisation in January may have led to a reduction in denitrification and elevated DNRA rate. Whilst the effect on denitrification in this study is not statistically conclusive, it must be acknowledged that this may be a factor of the experimental constraints and therefore should not be ignored. Despite the higher denitrification rate, the efficiency of denitrification is actually lower at the cage sites because the rate of nitrogen removed when compared to the rate of nitrogen returning to the water column (efflux of ammonium) is much lower.

At the control sites, denitrification rates were low and the process was depending more on nitrate from the sediments via nitrification (coupled nitrification-denitrification) rather than on nitrate from the water column (also commonly referred to as uncoupled nitrification-denitrification) which coincides with lower organic loading. The greater uptake of nitrate by the sediments at the cage and 50 m sites corresponds suggests that

denitrification in these organically enriched sites is more dependent on nitrate from the overlying water rather than the nitrate produced through the process of nitrification in the sediment. The higher uncoupled nitrification-denitrification rates over coupled nitrification-denitrification rate in the sediment at both the fish cages and 50 m sites could also reflect a reduction in diffusion distance for nitrate between the overlying water column and the anoxic denitrification zone in the sediments (Christensen et al 1989; Christensen et al 2000) due to the reduction in oxygen penetration depth from enhanced consumption of oxygen (Cai & Sayles, 1996). It is also possible that the ammonium released from the sediments is converted to nitrate in the water column via nitrification and this in turn stimulates uncoupled nitrification-denitrification in the sediment.

Ammonium effluxes were significantly higher in sediments taken from the fish cages than the controls. The flux rates of ammonium have been shown to be highly correlated with DIC production and oxygen consumption, as rapid mineralisation occurs during organic loading (Findlay & Watling, 1997). The increase in ammonium efflux may also reflect that nitrification processes were inhibited due to lack of oxygen. Other than that, DNRA which produces ammonium may be stimulated by the presence of sulfides (An & Gardner, 2002; Joye & Anderson, 2008). Higher rates of DNRA compared to denitrification under fish cages were observed by Christensen et al. (2000). In Macquarie Harbour, this observation was noted at Central lease but not at the Gordon and Liberty Point leases; this may be attributed to the lower organic enrichment levels at Gordon and Liberty Point and may reflect the different mineralisation rates at these sites compared to Central lease. The level of ammonium efflux associated with farm inputs in Macquarie Harbour was comparable with levels observed in salmon farming elsewhere (Nickell et al., 2003; Norđi et al., 2011). Ammonium is of particular interest in relation to eutrophication as it is readily taken up by phytoplankton and therefore has the potential to drive ecological change. The potential for

adverse effects would probably be limited by the low primary productivity previously reported for Macquarie Harbour (Edgar et al., 1999).

It has been suggested that organic enrichment can also modify the phosphorus cycle (Adrieux-Loyer et al 2014) and as a result changes in phosphate flux might provide an indication of farming impact. Aquaculture activities tend to increase the amount of phosphorus into the sediment through feed (Holby & Hall 1991; Wu 1995; Wang et al 2012). Phosphate is controlled by sediment mineralisation (Holmer et al 2003) and under oxic conditions, phosphate from the overlying water column and sediments will bind with $\text{Fe}(\text{OH})_3$ causing an uptake of phosphate, which would appear to be the situation at the control sites and most of the 50 m sites. However, under anoxic conditions $\text{Fe}(\text{OH})_3$ reduction occurs and the available Fe^{2+} binds to sulfides derived from sulfate reduction to form FeS and FeS_2 , thereby reducing the adsorption sites for phosphate (Sundby et al 1992; Andrieux Loyer et al 2014). Given that fish farming activity will result in highly reducing (sulfidic) conditions it is likely that iron will be limited and any excess phosphorus would be released, this could then be available to enhance primary productivity. In the current study phosphate fluxes were elevated at the cage sites but the impact of this is not known.

Whilst there were some clear differences in the sediment condition and nutrient processing at the farm, 50 m and control sites, there was little evidence of any major temporal differences in sediment processes with a couple of notable exceptions at the cage sites. The insignificant temporal differences particularly at the control sites indicates that the bottom water condition was relatively stable at these sites and most likely reflecting the condition throughout the harbour. The most notable temporal differences was the nutrient flux rates under cage 2 on Central lease which were higher in January and this could be due to the different stages of farming activity such as fallowing or stocking.

Benthic recovery following fallowing

Fallowing is the key mechanism used by farmers to manage the condition of the sediments beneath their farms. In the Southeast of Tasmania the fallowing period before restocking after each production cycle is generally about 3 months (Macleod et al., 2006). However, during this study period the regime in Macquarie Harbour is still being established, some cages have been left fallowed for considerably longer than 3 months. This study looked at the differences in benthic response associated with fallowing and stocking in Macquarie Harbour with a view to providing some understanding of how different regimes affected sediment performance and whether there was any variation within the harbour.

There were different stocking/ fallowing regimes at each of the study sites. When samples were collected in November and January the cages at Central lease were stocked, summer being the peak season for salmon growth (Battaglione et al., 2008), and when samples were collected in May and September the cages were fallowed. In contrast Gordon lease was stocked in September and fallowed when samples were collected in November to January and Liberty point was stocked in November and fallowed in January. Central lease, which is closer to the lower estuary, presented the expected response during stocked and fallowed period with the benthic flux rates increasing at both cages over the production cycle and decreasing during the fallowing period (hence the sediments showed signs of recovery). A similar pattern was also observed at the Liberty Point lease. The effect of fallowing at the Gordon lease was less clear. Benthic flux rates at Cage 2 (Gordon lease) increased during fallowing but decreased when the cage was stocked. Surprisingly, Cage 1 (Gordon lease) benthic nutrient fluxes declined when it was apparently stocked. Based on benthic flux rates, the benthic response during fallowing differed between the Central and Gordon leases.

These differences in benthic response associated with fallowing and stocking in Macquarie Harbour could be attributed to bottom water dissolved oxygen concentration throughout the year and/or hydrodynamics such as current flow and velocities which differed between the leases. This has the potential to influence the stocking/ fallowing response, there was an overall decrease in benthic flux rates during May and September in the Central and Gordon sites that could be a result of the bottom water dissolved oxygen concentrations being lower than in November and January. This reasoning is in line with findings presented in a recent report looking specifically at changes in bottom water dissolved oxygen in Macquarie Harbour (e.g. MHDOWG, 2014). Whilst, these interactions are not clear and more work is needed to establish the most appropriate fallowing regimes for sites in Macquarie Harbour it is clear that fallowing improved benthic conditions at Central lease and therefore that fallowing still should be considered as a beneficial management practice.

Conclusion and broader implications

This study has provided the first assessment of how the sediments response to fish farming in Macquarie Harbour. The responses were similar to other benthic environments receiving organic enrichment from farming activities but the response is also dependent on the environment where farming takes place. In Macquarie Harbour, the long residence time may present challenges in terms of oxygen availability and subsequently nutrient cycling. As renewal of bottom water dissolved oxygen is slow, the increase in biological and chemical oxygen demands may pose a risk for hypoxia; a condition that has been observed in recent years.

As oxygen demand in the organic enriched sediments increases and oxygen concentrations decrease, processes such as nitrification are likely to be inhibited. The results indicate that this will cause a shift from coupled nitrification-denitrification to uncoupled nitrification-denitrification. This demonstrates the importance of uncoupled nitrification-denitrification in Macquarie Harbour as a pathway for the removal of nitrogen from the system, especially in areas receiving higher organic loading. However, it must be noted that the rate of nitrogen returning to the water column under the cages exceeds the rate of removal through denitrification and the future implication of this is still unknown.

The variability in terms of the response towards fallowing and stocking and the unknown causes of this suggest that further investigation is required to understand the drivers of sediment function under the cages which could improve farming management practices in terms of stocking and fallowing regimes in Macquarie Harbour.

Chapter 4: Discussion

General Discussion

A great deal of our understanding of benthic nutrient processes is derived from studies undertaken in northern hemisphere temperate estuaries which by large differ from the estuary in this study. Firstly, Macquarie Harbour has dark tannin water and as a result benthic primary production is likely to be low due to limited light penetration (Edgar et al., 1999). Secondly, due to a shallow entrance at the lower reaches of the harbour, water residence time is long (DPIPWE et al., 2011; Koehnken, 1996). Together with water column stratification and other physical and biological characteristics, this makes Macquarie Harbour a rather unique estuary, more akin to a fjord system.

In this study, spatial variation in important benthic nutrient cycling processes and the potential environmental drivers in Macquarie Harbour were initially investigated (Chapter 2). Comparable to many other studies, dissolved oxygen (DO) was found to be important in regulating benthic nutrient cycling in the harbour and was most likely the primary driver of spatial variation in benthic respiration and nutrient fluxes. In areas where bottom water DO levels are particularly low, anaerobic respiration is an important pathways for organic matter mineralisation. Moreover, the low DO levels in the sediments appear to inhibit sediment nitrification (Caffrey et al., 1993; Joye & Anderson, 2008) in the harbour. As a consequence, much of the ammonium produced following the mineralisation of organic matter is released back into the water column.

Despite, the reduction in sediment nitrification, denitrification was recorded throughout the harbour. The uptake of nitrate into sediments across the study sites clearly demonstrated that the removal of nitrogen from the system via denitrification requires nitrate to be sourced from the water column in the low bottom water oxygen environment; this is commonly referred to as uncoupled nitrification-denitrification. Nitrification of ammonium

in the water column is hypothesised as the source of this nitrate (Cornwell et al., 1999; Herbert, 1999; Rysgaard et al., 1994). Importantly though, not all nitrate taken up by the sediment is being used for denitrification. Dissimilatory nitrate reduction to ammonium (DNRA), another process in the nitrogen cycling occurring in Macquarie Harbour sediments, also competes with denitrification for nitrate (An & Gardner, 2002) and this is probably due to the presence of sulfides (An & Gardner, 2002; Joye & Anderson, 2008). This process contributes to the production of ammonium. Another observation made in this study is that the sediments in the harbour generally adsorb phosphate.

The rapid growth of the aquaculture industry in Macquarie Harbour has led to the need for sustainable development to ensure that both industry and environment provide long term economic gain and environmental protection. Understanding the response of Macquarie Harbour sediments to organic enrichment was the next objective of this study (Chapter 3). Waste from fish farming such as uneaten feed and fish faeces are inevitably deposited onto the sediment (Pearson & Black, 2001; X. Wang et al., 2012) causing high levels of total carbon and nitrogen in the sediments as seen in this study. As shown in the conceptual diagram of Macquarie Harbour (Figure 19) such high and labile organic loading onto the sediment lead to elevated benthic respiration, especially through anaerobic pathways. As oxygen is most likely to be rapidly consumed by bacteria, nitrification efficiency is further reduced in areas of enrichment, thus leading to a greater release of ammonium into the water column. This is commonly observed in sediments under the influence of organic loading from fish farms (Hall et al., 1992; Hargrave et al., 1993; Holmer et al., 2005). Denitrifying bacteria must then heavily rely on nitrate from the water column to remove excess nitrogen from the sediments Figure 19. Although denitrification rates were observed to increase in farm sediments, the proportion of nitrogen mineralised that is denitrified is significantly lower in farm sediments given the much higher rates of respiration and ammonium release.

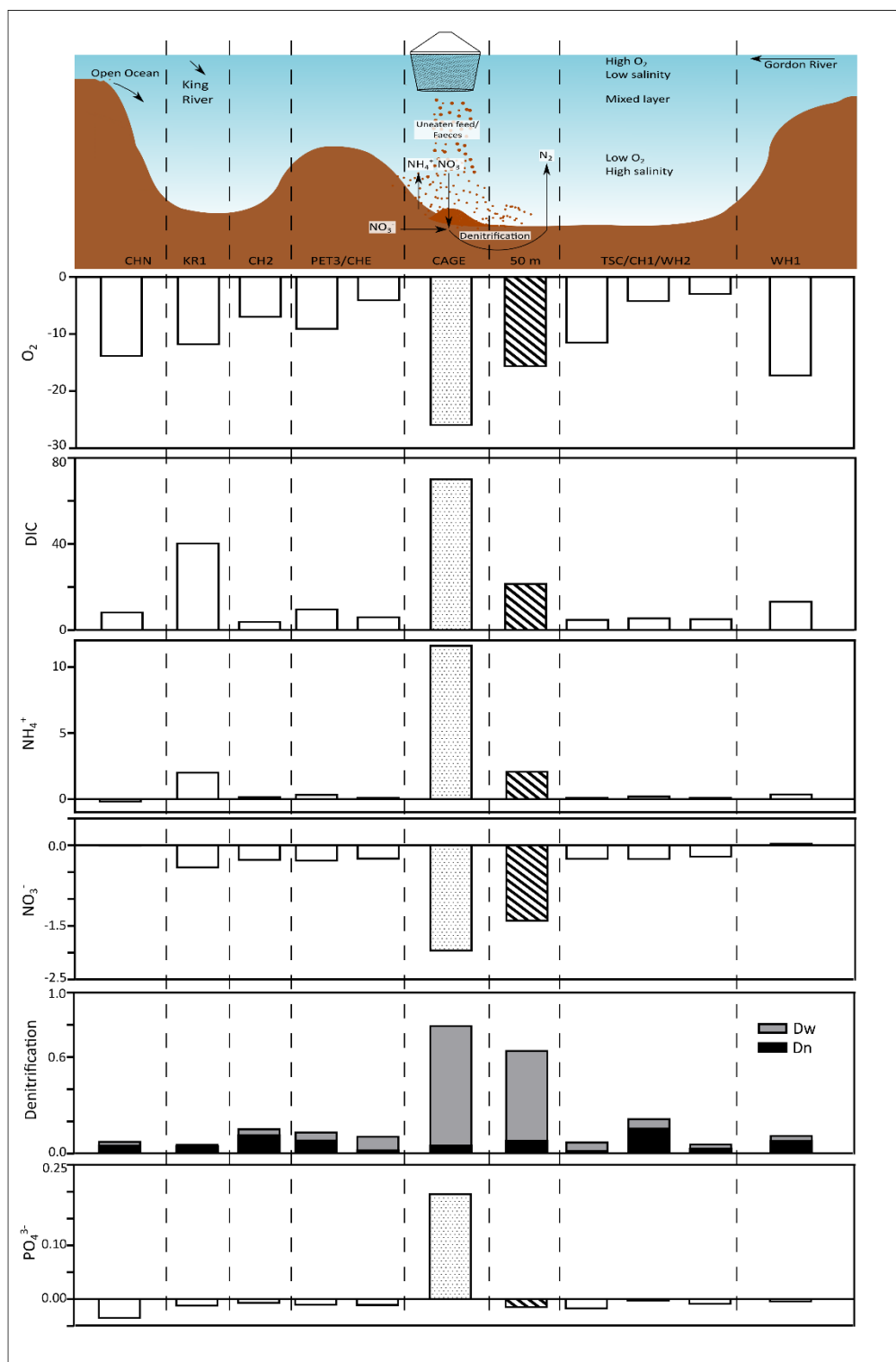


Figure 19 Conceptual diagram showing the benthic flux rates of oxygen (O₂), dissolved inorganic carbon (DIC), ammonium (NH₄⁺), nitrate (NO₃⁻), denitrification, and phosphate (PO₄³⁻) across Macquarie Harbour. All rates are presented in mmol m⁻² d⁻¹. All measurements were taken from November survey. To represent the cage and 50 m sites flux rates, the measurements from all six cage sites and three 50 m sites were averaged respectively.

The interesting finding in Macquarie Harbour is that despite the release of ammonium from sediments, particularly at the enriched sediments, environmental monitoring of the harbour showed no evidence of increased ammonium concentration in the overlying bottom waters over the period of rapid industry expansion (see MHDOWG, 2014). The most probable explanation is that the ammonium released from the sediments may be converted to nitrate in the water column via nitrification where oxygen availability is higher than in the sediments. Similarly, there has been no observable increase in nitrate concentrations in bottom waters over this period. Given the limited mixing and long residence time of bottom waters and the lack of light for primary production, denitrification in sediments may be responsible for removing the nitrogen, and thus buffering the system to the increased supply of nutrients to bottom waters due to farming. This remains to be tested and greater replication of process measurements in space and time are required.

This study has also shown that the effect of organic loading often extends to 50 m from farmed cages, however to what extent enrichment and benthic response extends beyond 50 m remains unknown. In this study, the sediment response to a decrease in organic enrichment during fallowing was also investigated. There were changes in the flux rates during fallowing that were consistent with expectations (i.e lower rates of respiration and release), but not at all sites. This suggests that there are other factors, such as bottom water DO levels during fallowing, that regulates the benthic processing of organic matter.

Conclusion

The harbour wide survey gave background flux rates and insights to some of the benthic processes occurring in the sediments in Macquarie Harbour while surveys at the fish farm sites showed the sediment response towards organic loading. Dissolved oxygen was identified as the key driver to most of the benthic processes in the sediments. The constant increase in biological and chemical oxygen demand in the water column and sediment may increase the chances for developing persistent hypoxia (Rabalais, 2009) when the harbour has already experiencing periodic hypoxia in recent years (Chapter 2 and Chapter 3).

This different sediment response towards the increase and decrease in organic enrichment at different areas of the harbour would require further investigation to fully understand the driver of this differences and the implication towards farm management. And given the importance of dissolved oxygen to the benthic processes such as nitrogen cycling, environmental managers have to ensure that the benthic oxygen demand does not exceed the rate of oxygen renewal in the harbour.

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